Psychobiological Correlates of Perceived Stress, Depressive Mood and Anxiety during Pregnancy

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Abbreviations

ADHD	Attention deficit and hyperactivity disorder
ANOVA	analyses of variance
AC	avoidance coping
ACTH	adrenocorticotropin-releasing hormone
CISS	Coping Inventory for Stressful Situations
CRH	Corticotropin-releasing hormone
CRI	Coping Responses Inventory
EC	Emotional coping
GAS	Birth Anxiety Scale
HPA	Hypothalamus-pituitary-adrenal axis
IUGR	Intraueterine growth retardation
LOD	Limit of detection
NuPCI	Revised Prenatal Coping Inventory
PCI	Prenatal Coping Inventory
RIA	Radioimmunoassay
SER	Stress experience during rest condition
SEA	Stress during amniocentesis
SEW	Stress experience caused by waiting for the results
SENR	Stress experience caused by the possibility of a negative result
STAI	State-trait Anxiety Inventory
TC	Task coping
UCL-19	Utrecht Coping List
UCN	Urocortin
WCQ	Ways of Coping Questionnaire
WOG	Weeks of gestation

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1. Introduction

Pregnancy is a very delicate phase in life, not only for the life of the child to be, but also for the future mothers. Significant changes go on in the body and also psyche of a pregnant women, including endocrine changes in concentrations of sex steroids and hormones of the hypothalamus-pituitary-adrenal axis (HPA), bodily changes, relationship adjustments, concerns of future parenthood or about the health of the child and many more.

Various factors can interrupt the fragile development of a new life, such as abuse of legal or illegal drugs, exposure to hazardous and harmful substances or malnutrition, just to name a few. The negative consequences of the above mentioned factors have been investigated very carefully and intensively. A relatively new field of research addresses the psychosocial variables and their biological correlates that might influence human pregnancy in either a positive or a negative way.

It has been shown that the psychological wellbeing of the pregnant women not only has an influence on the course of the ongoing pregnancy and the characteristics and outcome of birth, but on the wellbeing of the child and the mother pre-, peri- and postpartum as well. The new research field of fetal programming shows how enduring the impacts of maternal stress can be and how important it is to elucidate the pathways in order to get a clearer understanding. This could further on lead to the development of prevention campaigns programs.

On the one hand the present thesis explores the physiological background and correlates of perceived acute stress, (birth) anxiety and depressive mood during pregnancy. Several biomarkers have so far been identified which are clearly connected to the perception of stress, anxiety and depressive mood but which also seem to have an impact on pregnancy and birth complications and outcomes. Some of the biomarkers, especially the corticotropin-releasing hormone and urocortin also play an important role in maintaining healthy pregnancy and initiating birth at the right gestational age. But as in many other fields it’s the dose - and in this case also the timing that makes the difference between health and illness. If the levels of the above-mentioned peptides are too high in an early stage of pregnancy, the consequences might be pregnancy complications such as preeclampsia or preterm birth. If the levels of stress-related hormones are too high during pregnancy, the consequences might be as severe

as postpartum depression in the mothers and severe health consequences and altered HPA activity in the children.

On the other hand, this thesis investigates and evaluates the way pregnant women face stressors during pregnancy. Coping can directly or indirectly (buffering effects) affect personal wellbeing. Unfortunately, the effects of coping during pregnancy are still poorly understood. The use of effective coping styles can have protective effects when it comes to adversities, but ineffective coping styles, on the contrary, can be harmful and aggravate the effects of adversities such as stress, anxiety and depression.

The field of psychosocial variables and their biological correlates is wide and this thesis addresses only a small area of this spectrum. Further research is necessary to give pregnant women the best possibilities for healthy pregnancies full of wellbeing and joy.

Part I. Theoretical background

In this section, the background of psychosocial and biological variables associated with stress and psychological wellbeing is depicted. All of these variables have numerous roles and effects in the human body. Here, the description is limited to their main effects and the focus is set on their specific roles and impacts during pregnancy.

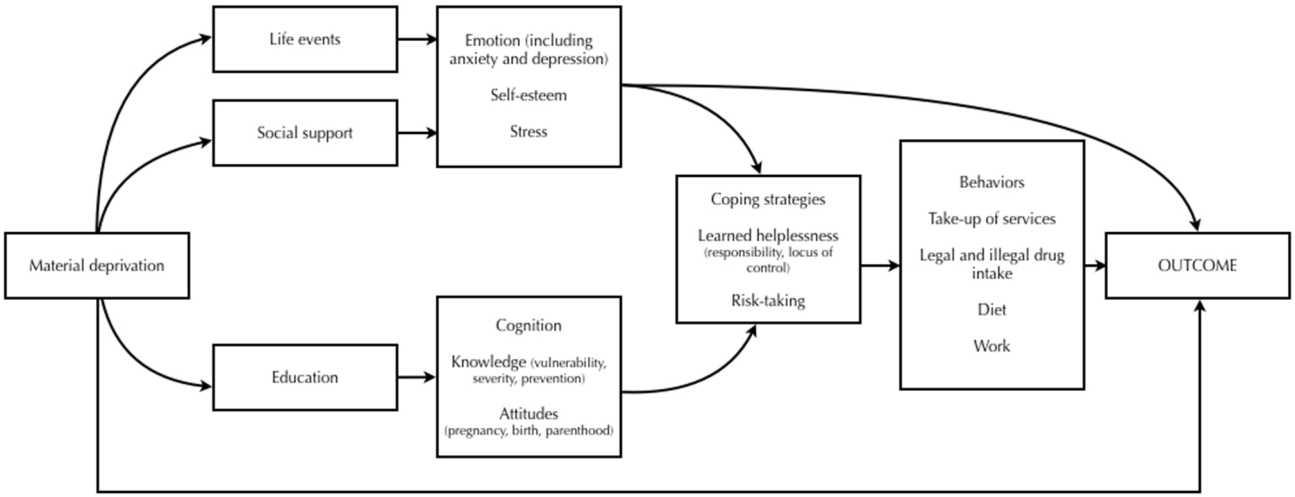
The main objective of this section is to clearly show that psychosocial and biological variables are inseparably interwoven, each side having impacts on the other side, each being stimulus and outcome simultaneously. Their interplay should thus be in the scope of future research.

2. Stress and the psychophysiological stress response during pregnancy

While the impact of harmful factors such as malnutrition, drugs, smoking, alcohol, teratogens and other chemicals on pregnancy and the unborn baby have been widely studied in the past, the study of the impact of psychosocial variables is a relatively newly emerging field of research. It has recently been shown that not only lifestyle and environmental factors may have severe and long term effects on the mothers-to-be, the pregnancy, the birth and the babies, but also psychological well-being during this intense period of time for a women may leave it's traces (Dunkel-Schetter, 2011).

Psychological wellbeing and the psychological and biological effects of stress are closely related (Dunkel-Schetter, 2011). The focus of this thesis will be on stress, starting with a short digression on the basics of stress while then focusing on the effects of stress during pregnancy, coping with stressors during pregnancy, as well as prenatal anxiety and depressive mood. All these factors have been associated with complications during pregnancy and adverse pregnancy outcomes in previous research. Thereby, direct and indirect effects have been suggested, as seen in Figure 2.1, adapted from (Hoffman & Hatch, 1996).

Figure 2.1. Stress, coping, anxiety and depression during pregnancy, adapted with permission[1] (originally by Hoffman & Hatch, 1996)



Note. Pathways of the influences of stress, coping, anxiety and depression during pregnancy

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2.1. The human stress response during pregnancy

To understand how possible stressors may affect pregnant women, a short discourse on stress and the human stress response will be given first.

2.1.1. Discourse on the basics of the human stress response

The term "stress" and "stress response" are usually mentioned with a negatively colored, bitter aftertaste, even though stress can be provoked in either pleasurable (also called eustress) or adverse (also called distress) situations (Dorn & Chrousos, 1993; Stratakis & Chrousos, 1995). Still, in common language, "I am stressed" mostly stands for a feeling of decreased well-being in the speaker, one of not feeling at ease and being somewhat out of the balance. What commonly gets forgotten is the evolutionarily positive value of the stress response in consideration of the survival of a) the individual and b) the species (Stratakis & Chrousos, 1995) – a normal functioning of our reaction to one or multiple stressor is indispensable for our physical and mental health.

Stress is what we encounter when our highest good – physical and mental homeostasis – is threatened by external or internal stressors, a state that we immediately try to reestablish with help of physiologic and behavioral adaptation responses (Chrousos, 1998). Homeostasis is also known as allostasis and any disturbance of it as allostatic load (McEwen, 1998); the actions of the stress response are necessary to stabilize the system.

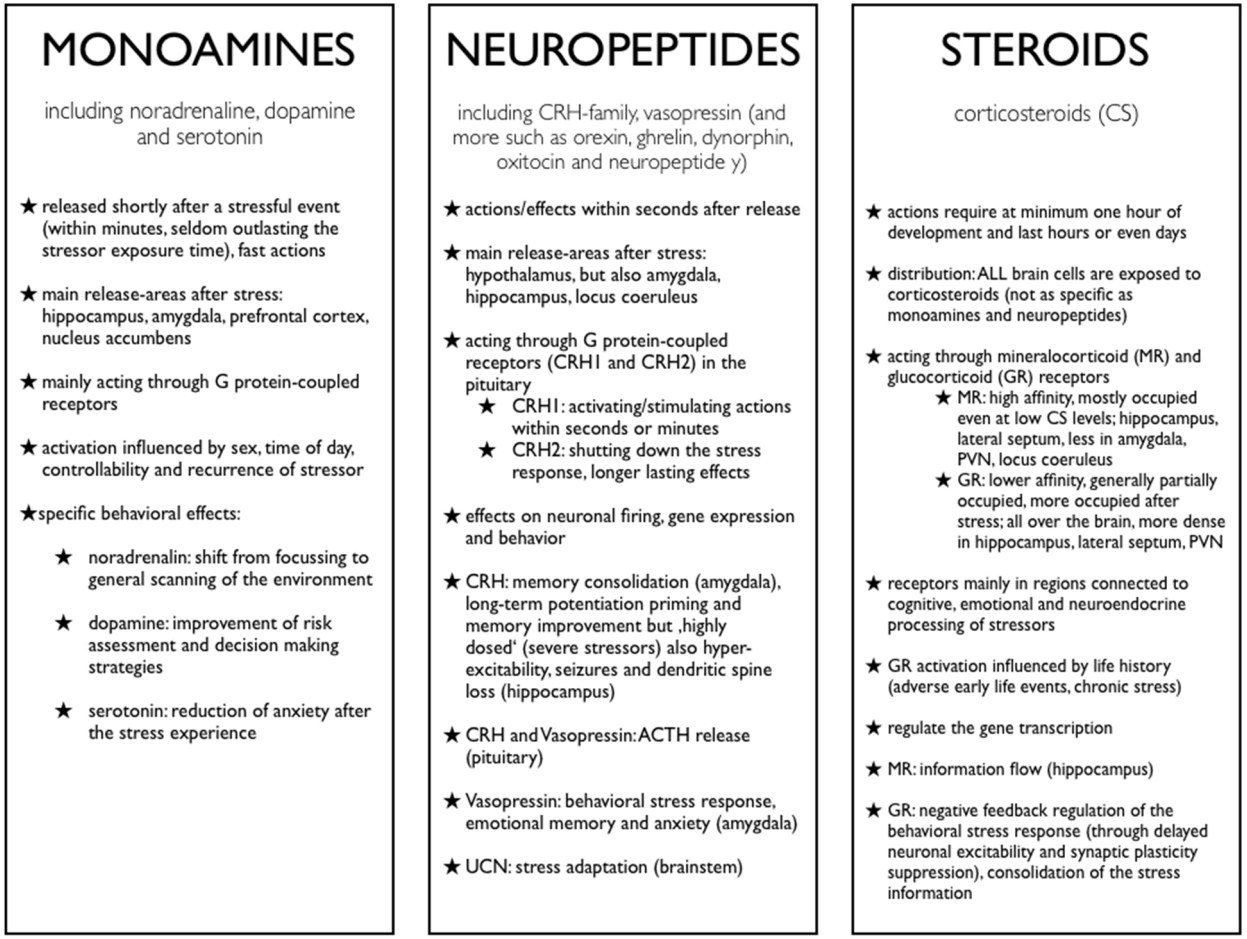
Stress is evolutionary explained by preparation for fight or flight; the adaptive responses therefore include increased vigilance and arousal, heightened analgesia, suppression of appetite and the reproductive system on the behavioral side and a redirection of oxygen and nutrients, increased heart rate, blood pressure and respiration rate and an increased glucogenesis and lipolysis on the physiological side (Charmandari, Tsigos, & Chrousos, 2005) – preparing the whole organism, so to speak.

The biological stress response basically contains of two separate pathways in the sense of two temporal waves (Joëls & Baram, 2009): The first - or fast - wave acts through the activation of norepinephrine, dopamine, CRH (through the CRH-1-receptor) and serotonin and is responsible for all the fast stress responses such as increased vigilance, appraisal of the situa-

tion and the following choice of strategy (fight or flight) to handle the situation (Joëls & Baram, 2009). These quick responses, evolutionarily spoken, guarantee survival (of the fittest), but they do not account for the long-term adaption to stressors. A slower, second wave of the stress response action is mainly mediated by corticosteroids (through glucocorticoid receptors) and the CRH-2-receptor (Joëls & Baram, 2009), altering gene expression processes and cell functioning. The second wave is responsible for the restoration of homeostasis and the consolidation of stress-related information. A more detailed description of the actions of steroids, monoamines and neuropeptides can be found in Figure 2.2, which is mainly based on Joëls & Baram, 2009[2].

[2] It secondly refers to some of their references on monoamines (Amat et al., 2005; Goto, Otani, & Grace, 2007; Linthorst & Reul, 2008; Maier & Watkins, 2005; Mitsushima, Yamada, Takase, Funabashi, & Kimura, 2006; Morilak et al., 2005; Piazza et al., 1996), neuropeptides (Adamec, Holmes, & Blundell, 2008; Aldenhoff, Gruol, Rivier, Vale, & Siggins, 1983; Aston-Jones & Cohen, 2005; Blank, Nijholt, Eckart, & Spiess, 2002; Chen et al., 2004; Chen, Bender, Frotscher, & Baram, 2001; Chen, Dubé, Rice, & Baram, 2008; Ehlers et al., 1983; Gallagher, Orozco-Cabal, Liu, & Shinnick-Gallagher, 2008; Harbuz et al., 1992; Koob, 2008; Landgraf & Neumann, 2004; Lee et al., 2000; Merali, Khan, Michaud, Shippy, & Anisman, 2004; Roozendaal, Brunson, Holloway, McGaugh, & Baram, 2002; Stenzel-Poore et al., 2000; Swanson, Sawchenko, Rivier, & Vale, 1983; Valentino & Van Bockstaele, 2008; Wang, Wayner, Chai, & Lee, 1998) and steroids (Champagne et al., 2008; Joëls, Karst, Krugers, & Lucassen, 2007; Kim & Diamond, 2002; Lu et al., 2006; McGaugh, 2004).

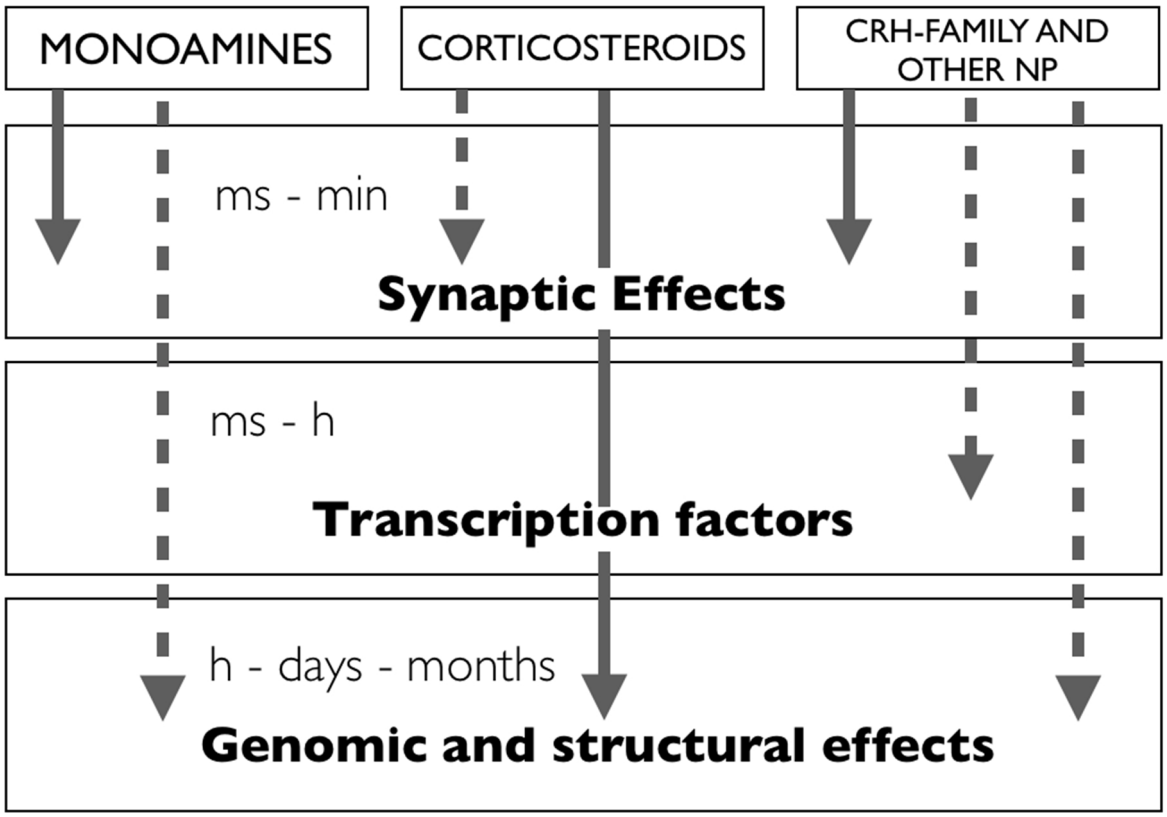
Figure 2.2. Monoamines, neuropeptides and steroids and their role in the stress reaction



Note. The short- and long-term effects and pathways of monoamines, neuropeptides and steroids during the human stress response. CRH = corticotropin-releasing hormone; CHR1 = CRH-1-receptor; CRH2 = CHR-2-receptor: ACTH = adrenocorticotropin-releasing hormone; UCN = urocortin; CS = corticosteroids; MR = mineralocorticoid receptor; GR = glucocorticoid receptor; PVN = paraventricular nucleus

The first-wave-second-wave differentiation is a raw classification. The first wave is associated with the sympathetic nervous system (activating the adrenal medulla where epinephrine and norepinephrine are released as consequences) (Joëls & Baram, 2009), while the second wave is associated with the activation of the hypothalamus-pituitary-adrenal axis (HPA), where a cascading reaction takes place. CRH is released by the hypothalamus and stimulates the pituitary to release adrenocorticotropin-releasing hormone (ACTH), which in turn stimulates the release of cortisol in the adrenal cortex (Tsigos & Chrousos, 2002). The system is controlled by a negative feedback mechanism by ACTH and cortisol inhibiting CRH release of the hypothalamus (Charmandari et al., 2005). Beyond these two waves, corticosteroids have their short-term actions as well, and so do monoamines and neuropeptides such as those belonging to the CRH-family, as seen in Figure 2.3.

Figure 2.3. Short- and long-term effects of monoamines, corticosteroids and neuropeptides in the stress response, adapted with permission[3] (originally by Joëls & Baram, 2009)



Note. Primary effects in strait, secondary effects in dotted lines.

[3] Adapted by permission from Macmillan Publishers Ltd: Natures Reviews Neuroscience: Joëls, M. & Baram, T. Z. The neuro-symphony of stress. *Nature reviews neuroscience*, *10*(6), 459–466, copyright 2009. http://www.nature.com/nrn/journal/v10/n6/full/nrn2632.html

The proper functioning of the stress response depends on a fine tuned orchestrating of all participants (Joëls & Baram, 2009). Malfunctioning in the sense of hypo- or hyper-responsiveness of the HPA is associated with various physical and mental illnesses and disorders (Stratakis & Chrousos, 1995). States of decreased and increased HPA are depicted in Table 2.1. But an alteration or adaptation may also serve as a preparation of the body to a special state, in connection with the various other important roles of the HPA-related hormones. In practice, HPA-hormones undergo specific changes during pregnancy due to the fact that several reproductive tissues, especially the placenta, start producing CRH as well in much higher amounts than the pituitary (Thomson, 2008). These characteristics will be described in the following, with an emphasis on the hormones in Chapter 2.2, *The biological stress axis and its hormones*, *especially corticotropin-releasing hormone and urocortin during pregnancy*, of this thesis.

Table 2.1. States and illnesses associated with dysfunctional HPA axis activity, adapted with permission[4] (originally by Stratakis & Chrousos, 1995).

Increased HPA axis activity	Decreased HPA axis activity
Chronic stress	Adrenal insufficiency
Melancholic Depression	Atypical/seasonal depression
Anorexia nervosa	Chronic fatigue syndrome (CFS)
Obsessive compulsive disorder (OCD)	Fibromyalgia
Panic disorder	Hypothyroidism
Excessive exercise	Nicotine withdrawal
Chronic active alcoholism	Post-glucocorticoid therapy
Alcohol and narcotic withdrawal	Post-Cushing syndrome
Diabetes mellitus	Postpartum period
Central obesity (metabolic syndrome X)	Post-chronic stress
Sexual abuse	Rheumatoid arthritis
Hyperthyroidism	
Premenstrual tension syndrome	
Cushing syndrome	
Pregnancy	

Note. States and illnesses in connection with increased or decreased HPA axis activity.

2.2. The hypothalamus-pituitary adrenal axis (HPA) during pregnancy with a special focus on CRH

Among the various changes that the female body goes through throughout pregnancy, there are also changes in the stress response, which adjust to this phase of life. Moreover, aside from their key role in the HPA and, in consequence, the human endocrine stress response, CRH and cortisol do play an important role in pregnancy (Kalantaridou, Makrigiannakis, Zoumakis, & Chrousos, 2004; Lindsay & Nieman, 2005) and fetal development (Ishimoto & Jaffe, 2011; Murphy, 1981). The changes of the HPA and especially CRH and UCN will be

[4] Permission granted by John Whiley and Sons via Rightslink. Licensed content publication: Annals of the New York Academy of Sciences. Licensed content title: Neuroendocrinology and Pathophysiology of the Stress System. Licensed content author Constantine A. Stratakis, George P. Chrousos. Licensed content date: 2006. © New York Academy of Sciences. http://onlinelibrary.wiley.com/doi/10.1111/j.1749-6632.1995.tb44666.x/abstract;jsessionid=CB3D05582DB07106907F4E9FB3FCA1F2.f03t04

described in the following.

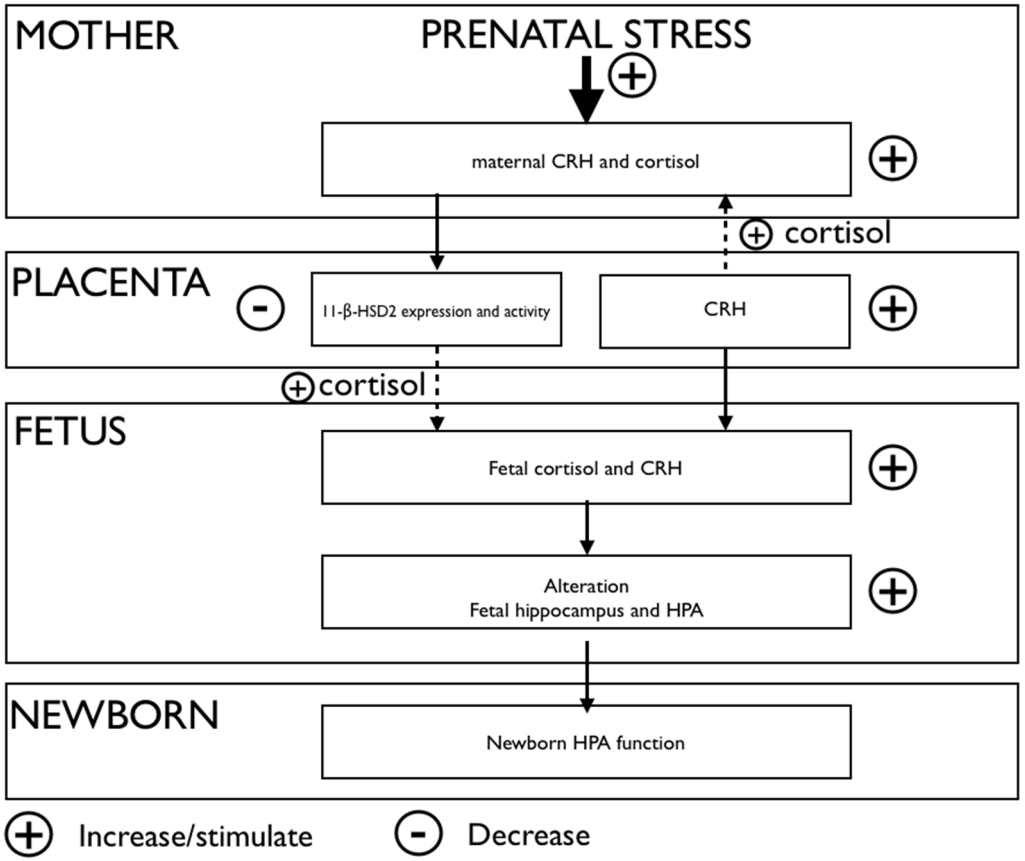
The levels of HPA-related hormones, including CRH, ACTH and cortisol are progressively rising during the course of normal pregnancy (de Weerth & Buitelaar, 2005; Mastorakos & Ilias, 2003). It has to be noted that pregnancy-related increases in CRH (Chen et al., 2010), ACTH (Lindsay & Nieman, 2005) and cortisol (Allolio et al., 1990) also lead to difficulties in disease detection (and treatment) of illnesses such as the Cushing's Syndrome (Lindsay & Nieman, 2005) during gestation. The progressive increases can be accounted for by the fact that reproductive tissues and mainly the placenta start producing CRH in large amounts in pregnancy: The CRH levels, peaking shortly before birth, increase progressively especially from the second trimester onward reaching a thousand fold of the level in non-pregnant women (de Weerth & Buitelaar, 2005). Moreover, most of the maternal plasma CRH derives from CRH from the placenta (Chen et al., 2010). Placental CRH in turn stimulates local ACTH secretion (Petraglia, Sawchenko, Rivier, & Vale, 1987).

The placental CRH released by the placenta is , in general, indistinguishable from hypothalamic CRH (Stalla et al., 1989) and is as well mediated by the maternal HPA. Apart from this general similarity, however, it has one unique feature: While the HPA-CRH-release is down regulated by cortisol, the placental CRH-release is stimulated by cortisol at the same time, consequently up regulating the maternal HPA-system (King, Smith, & Nicholson, 2001), as seen in Figure 2.3.

Moreover, this progressively increasing CRH and cortisol levels influence the fetal HPA (Charil, Laplante, Vaillancourt, & King, 2010).

It has been suggested that in reaction to this massive increase in stress hormones, the anterior pituitary might develop a desensitization to (placental) CRH in order to prevent pathologically elevated ACTH and cortisol levels (Thomson, 2008). This suggestion goes along with the observation that general biological reactivity to a stressor seems to be dampened during human pregnancy (de Weerth & Buitelaar, 2005), especially during the third trimester shortly before birth, where one could even speak of a maternal hyporesponsiveness to stress (Slattery & Neumann, 2008). This hyporesponsiveness is a protective factor, as maternal excessive glucocorticoid releases during a stress response might disturb healthy fetal development (Slattery & Neumann, 2008).

Figure 2.4. Interaction of the maternal and fetal stress systems, adapted with permission[5] (originally by Charil et al., 2010)



Note. Prenatal maternal stress leads to higher maternal and fetal CRH and cortisol levels. While the hypothalamic CRH release is suppressed by cortisol (in order to shut the stress response down), the placental CRH release is increased by cortisol. This leads to a progressive increase of CRH and, in turn, cortisol. Moreover, prenatal stress leads to a decrease of release and activity of 11-β-HSD2, an enzyme acting as a protective mechanism by converting active cortisol into inactive cortisone. The down-regulation of 11-β-HSD2 therefore leads to an increase in cortisol exposure of the fetus. CRH = corticotropin-releasing hormone; 11-β-HSD2 = 11-beta-hydroxysteroid dehydrogenase type 2.

[5] Adapted with the permission from *Brain research reviews*, *65*(1), Charil, A., Laplante, D. P., Vaillancourt, C., & King, S. Prenatal stress and brain development. 56–79, Copyright (2010), with permission from Elsevier. http://www.sciencedirect.com/science/journal/01650173/65/1

2.3. Corticotropin-releasing hormone and urocortin during pregnany

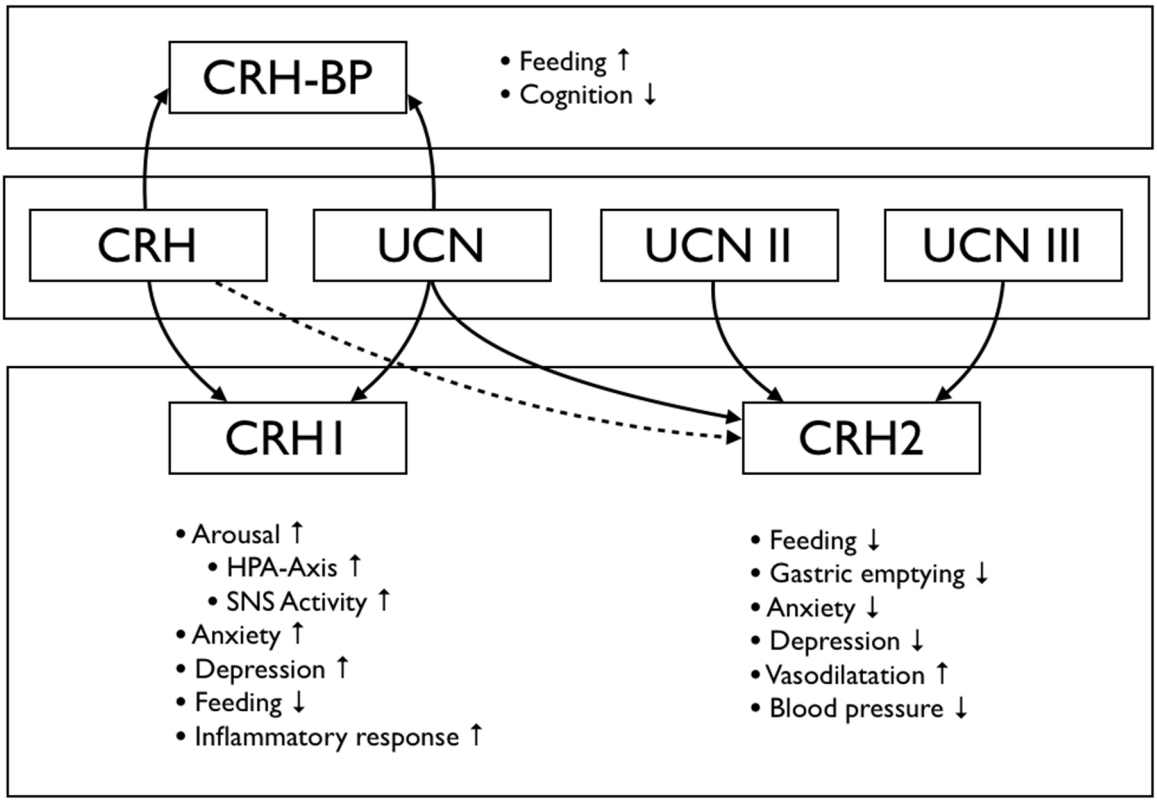
CRH and UCN derive from the same peptide precursor as that found as early as in metazoan ancestry (Lovejoy & Balment, 1999). Both peptides play various roles in psychosocial phenomena such as stress and anxiety (and their biological correlates) as well as a wide range of physiological assignments, for instance in vaso- and thermoregulation, metabolism, growth, locomotion and reproduction (Lovejoy & Balment, 1999). Specific up- or down-regulating effects over the CRH1- and/or CRH2-receptor are depicted in Figure 2.4. While CRH1-receptor bound actions are mostly arousing, the impacts acting via the CRH-2-receptor are mostly dampening or even calming (Bale & Vale, 2004; Dautzenberg & Hauger, 2002).

It has to be noted that apart from CRH and UCN or UCN I, as it is also called, two other peptides belong to the CRH-family: The stresscopin-related peptide or UCN II and stresscopin or UCN III (Dautzenberg & Hauger, 2002). As in the general literature, UCN I is referred to as UCN (without numerical extension) in the following.

For this thesis, two fields are especially interesting: The role of CRH and UCN in the endocrine stress response and during pregnancy.

The various peptides of the CRH-family do have important and distinct roles in the human stress reaction. CRH, as depicted above, works over the CRH-1-receptor and is stimulating the human stress response. UCN II and UCN III are specific ligands of the CRH-2-receptor and are considered to have an important role in dampening the stress sensitivity (Bale & Vale, 2004) or, as suggested in animal studies, in stress recovery (Neufeld-Cohen et al., 2010). UCN I might have a special role, as it binds to both CRH-1- and CRH-2-receptors, Bale and Vale (2004) and therefore may assume a dual role. The signaling pathways are depicted in Figure 2.4.

Figure 2.5. Paracrine and endocrine tasks of CRF and UCN, adapted with permission[6] (originally by Dautzenberg & Hauger, 2002)

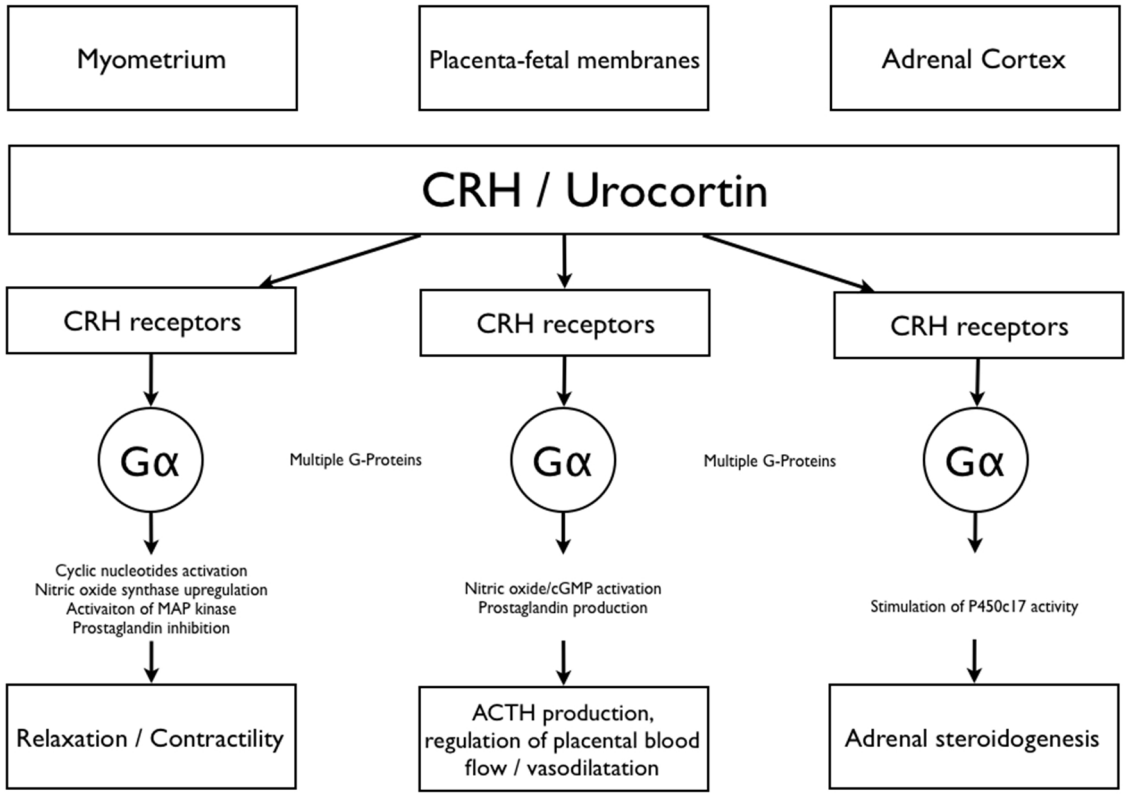


Note. ↑ stands for increasing/stimulating effects, ↓ stands for decreasing/inhibiting effects. CRH-BP = corticotropin-releasing hormone binding protein; CRH = corticotropin-releasing hormone; UCN = urocortin, UCN II = urocortin II; UCN II = urocortin III; CRH1 = CRH-1-receptor; CRH2 = CRH-2-receptor; HPA-axis = hypothalamus-pituitary-adrenal-axis; SNS = sympathetic nervous system.

A general overview of the specific tasks of CRH and UCN and the matching pathways is given in Figure 2.5.

[6] Adapted from *Trends in pharmacological sciences*, *23*, Dautzenberg, F., & Hauger, R. (2002). The CRF peptide family and their receptors: Yet more partners discovered. 71–77, with permission from Elsevier. http://www.sciencedirect.com/science/journal/01656147/23/2

Figure 2.6. CRH and UCN pathways during pregnancy, adapted with permission[7] (originally by Hillhouse et al., 2002)



Note. The pathways and effects of CHR and UCN derived from the myometrium, placental-fetal membranes and the adrenal cortex during pregnancy. Gα stands for multiple G-proteins. CRH = corticotropin-releasing hormone; ACTH = adrenocorticotropin-releasing hormone; Gα = guanine nucleotide binding proteins; MAP kinase = mitogen-activated protein kinase; cGMP = cyclic guanosine monophosphate; P450c17 = steroid 17-alpha-monooxygenase (cytochrome P450, family 17, subfamily A, polypeptide 1)

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http://www.reproduction-online.org/content/124/3/323.long

In the following, the roles and impacts of the two peptides during pregnancy will be elucidated separately. The above-depicted graph serves as a visual aid in understanding the pathways.

2.3.1. Corticotropin-releasing hormone

CRH is a peptide consisting of 41 amino acids (Bale & Vale, 2004). Its major role in the human stress response (Grammatopoulos & Chrousos, 2002), especially concerning the HPA, has already been described above. The particularity of the HPA during human pregnancy, namely the positive feedback loop of cortisol on placental CRH promoter activity is in contrast to the negative feedback on hypothalamic CRH promoter activity (King et al., 2001; Thomson, 2008) has as well been already described above. In the following, the focus will be on the specific role of CRH across pregnancy.

2.3.1.1 General functions of CRH during pregnancy

During pregnancy, CRH is, in addition to its presence at the usual bodily sites, also located in the placenta, the uterine decidua and the fetal membranes (Warren & Silverman, 1995). As Figure 2.5 shows, CRH is important: for relaxation and contractility of the myometrium (Grammatopoulos & Hillhouse, 1999), for its vasodilatory effects and therefore regulation of the placental blood flow (Bale & Vale, 2004), for its stimulation of ACTH secretion in the placenta (Petraglia et al., 1987) and for its steroidogenic activity in the fetal adrenal cortex (Bale & Vale, 2004). Its main role is seen as the "placental clock" (McLean et al., 1995): as CRH-levels rise during pregnancy, starting during the second trimester and reaching their peaks shortly before parturition while simultaneously CRH-binding protein levels start to fall (McLean et al., 1995; Thomson, 2008), a condition that leads to hyper-cortisolism in late pregnancy (Chrousos & Torpy, 1998).

CRH is also important for fetal (organ) maturation (McLean & Smith, 1999). A mediator role is suggested when it comes to fetal development: By stimulating fetal cortisol and DHEAS, it has again a function of timing with respect to fetal organ maturation in time for parturition (Fadalti et al., 2000; Majzoub & Karalis, 1999). This fact explains the sense of a positive feedback loop of cortisol on placental CRH, as cortisol is indispensable for the matu-

ration of fetal organs.

2.3.1.2. Relation to pregnancy complications and outcomes

2.3.1.2.1 CRH measured in maternal and umbilical cord plasma

The best investigated relationship is the one of elevated CRH-levels and preterm birth (Fadalti et al., 2000; Hobel, Arora, & Korst, 1999; Kalantaridou et al., 2010; Korebrits et al., 1998; Sandman et al., 2006; Schulkin, 1999). CRH is not only related to preterm labor (McLean & Smith, 2001) and birth, but to gestational length in general: as early as in the second trimester**,** CRH-levels might be able to distinguish women delivering pre-, post- and at term (McLean et al., 1995). Schulkin (1999) suggests that an over-expression of placental CRH is an indicator of an endangered pregnancy and that the resulting (preterm) labor is a protective mechanism, in the sense of reaction to allostatic overload. This is in line with other hypotheses of preterm birth as an adaption to poor conditions and insufficient extra- and intrauterine environments (Pike, 2005).

Apart from being the placental clock, CRH is related to complications during pregnancy, suggesting that higher maternal CRH levels are associated with preeclampsia (Kalantaridou et al., 2010; McLean & Smith, 2001) and intrauterine growth retardation (IUGR) of the fetus (Kalantaridou et al., 2010; McLean & Smith, 2001). Both of these complications are related to placental dysfunction (Kalantaridou et al., 2010). The association of highly elevated CRH-levels and IUGR is found not only in maternal plasma CRH, but also in umbilical cord plasma, whereas cortisol levels did not differ between IUGR- and normal pregnancies (Goland et al., 1993). Higher CRH-levels have also been found in placentas associated with spontaneous abortions (Minas et al., 2007).

Apart from physiological repercussions, other studies have demonstrated relationships between CRH-levels and the psychological wellbeing of the mothers postpartum. It has been shown that the high CRH-levels in late pregnancy are accompanied by a maternal hypercortisolism during the same period (Chrousos & Torpy, 1998). After birth, a relatively short subsequent suppression of CRH has been detected and the suggestion has been made that this suppression may be correlated with postpartum blues, postpartum depression and certain auto-

immune diseases in this phase (Chrousos, 1999; Kalantaridou et al., 2010).

Many research groups suggested a mediating or moderating role of HPA-hormones when it comes to impacts of stress on pregnancy (see above). It has, however, to be noted that there are authors that doubt a relationship between the usual stress biomarkers and perceived stress assessed by common stress questionnaires during pregnancy (Harville, Savitz, Dole, Herring, & Thorp, 2009). This doubt has been strengthened by several studies that reported neither a relationship of maternal plasma CRH with perceived stress (Himes & Simhan, 2011; Kramer et al., 2009; Petraglia et al., 2001) nor an increase of CRH release into fetal plasma as a reaction to a stressor (Gitau, 2004).

2.3.1.2.2 CRH measured in amniotic fluid

The assessment of plasma CRH can be made relatively simply with maternal blood samples and has therefore been widely studied in plasma. So far, only a few studies examined amniotic fluid CRH levels (Florio et al., 2008; Menon, Arora, Hobel, & Fortunato, 2008; Salminen-Lappalainen & Laatikainen, 1990; Stalla et al., 1989; Torricelli, Voltolini, Galleri, Biliotti, Giovannelli, De Bonis, De Pascalis, Centini, et al., 2009). This might be due to the fact, that amniotic fluid sampling is ethically justifiable only for medical reasons, or a wish for prenatal diagnostics.

In the early years of these investigations, it was discovered that amniotic fluid CRH levels are similar to the umbilical cord plasma CRH levels, but twenty-fold lower than maternal plasma CRH levels (Salminen-Lappalainen & Laatikainen, 1990; Stalla et al., 1989). Amniotic fluid CRH has also been brought into connection with preterm birth, suggesting that elevated levels go along with preterm delivery (Menon et al., 2008), although other studies did not find these relations (Torricelli, Voltolini, Galleri, et al., 2009). Higher amniotic fluid CRH levels were also found in women with intra-amniotic infections and inflammations at term (Florio et al., 2008).

2.3.1.3. Summary on CRH in human pregnancy

Whereas CRH levels in non-pregnant women are barely measurable, CRH-levels in pregnant women are high and mainly derive from placental CRH. As many roles CRH has in maintenance of pregnancy, fetal development and timing of parturition, as delicate are the mechanisms and as sensitive is the pregnant body to changes in concentrations of this peptide. Several pregnancy complications and adverse outcomes have been brought in connection with altered, mostly elevated CRH levels. Other than in non-pregnant women, a connection of CRH-levels and perceived stress could not be shown. Maternal plasma CRH levels are much higher than amniotic fluid CRH levels and the connections of the latter with complications during pregnancy and/or maternal stress are still poorly understood.

2.3.2. Urocortin

Urocortin, the first additional member of the CRH-family discovered, was initially described in 1995 (Vaughan et al.) and first synthesized in 1996 (Donaldson et al.). The peptide consisting out of 40 amino acids (Latchman, 2002) and has a 45% homology with CRH (Vaughan et al., 1995). There are two other members of the CRH-family, the stresscopin-related peptide (or urocortin II) and stresscopin (or urocortin III). These two close relatives bind to the CRH-1-receptor (Dautzenberg & Hauger, 2002). UCN (as mentioned above, urocortin I) binds to both CRH-1- and CRH-2-receptors. It binds to both of them more tightly then with CRH itself (Fekete & Zorrilla, 2007; Vaughan et al., 1995). As CRH, UCN is also highly affiliated to the CRH-binding protein (Florio, Vale, & Petraglia, 2004).

Due to its binding properties to the two CRH-receptors, the role of UCN in the human endocrine stress response is considered intermingling, as it binds to both CRH-1- (mostly stimulating/activating effects) and CRH-2-receptor (mostly inhibiting, decreasing effects). Its relevance may be focused on the dampening of stress sensitivity (Bale & Vale, 2004). Animal studies suggest a key role of all three urocortins in stress recovery but not in the acute stress response (Neufeld-Cohen et al., 2010). This latter fact seems surprising inasmuch as UCN is known to stimulate the HPA axis and consequently ACTH release from the pituitary (Fekete & Zorrilla, 2007). Moreover, it is suggested that UCN plays a role in the cardiovascular response to stress (Latchman, 2002).

UCN is located in the central nervous system (subcortical; its major site of synthesis is the Edinger-Westphal nucleus) as well as in the periphery, i.e. the heart, adipose tissue, skin, and in reproductive tissues, the placenta and the fetal membranes (Fekete & Zorrilla, 2007). Apart from the stress response, UCN has functions in osmoregulation, cardiovascular functioning (especially vasodilatory effects), the energy balance of the body – as well as energy expenditure, food intake, gastrointestinal functions, immune functions (as it is also located in immune system-related tissues such as the thymus and the skin), reproductive functioning, hearing and anxiety- and in depression-related behavior (Fekete & Zorrilla, 2007).

2.3.2.1. General functions of UCN during pregnancy

In a very early study (considering the year of the first detection of UCN), Petraglia et al. (1996) discovered that the placenta and gestational tissues express UCN, but in 1998, Glynn, Wolton, Rodríguez-Liñares, Phaneuf, and Linton stated that UCN is neither extensively translated in pregnancy-related tissues nor detectable in the plasma of pregnant women. It was only two years later that Clifton et al. (2000) revised these findings by studying the localization and characterization of the peptide during pregnancy. They were able to detect UCN in the maternal plasma as early as the 7th week of gestation onwards and revealed that the concentrations of the peptide did not change during the course of pregnancy, not even when labor started. More recent studies affirm the presence of UCN in pregnancy-related tissues and the consequent important role of the peptide (Fekete & Zorilla, 2007; Latchman, 2002).

Urocortin, together with CRH, seems to be responsible for the mechanisms leading to parturition (Iavazzo, Baka, & Malamitsi-Puchner, 2009; Kalantaridou et al., 2010), playing a key role in the contractility of the uterus (Florio et al., 2004; Petraglia, 1999). It is said to stimulate the placental ACTH secretion (Florio et al., 2004; Petraglia, 1999).

Moreover, a relation to placenta maintenance is suggested (Iavazzo, Baka, et al., 2009).

2.3.2.2 Relation to pregnancy complications and outcomes

It is hypothesized that urocortin may be related to preeclampsia (Iavazzo, Baka, et al., 2009) and a possible connection to preterm labor and preterm birth is currently being discussed (Iavazzo & Malamitsi-Puchner, 2010; Iavazzo, Tassis, et al., 2009; Torricelli, Voltolini, Galleri, et al., 2009).

2.3.2.2.1 Urocortin measured in amniotic fluid

Most of the recent studies on UCN in human pregnancy measure the peptide in amniotic fluid, in contrast to the wide tradition of measuring CRH in maternal plasma. The stable levels of UCN in maternal plasma may have contributed to the fact that researchers focus on the amniotic fluid levels of the peptide.

The structural and functional similarities of CRH and UCN suggest the importance of an investigation on the relationship of UCN with preterm birth. So far, there are controversial findings (Iavazzo & Malamitsi-Puchner, 2010). While Torricelli, Voltolini, Galleri, et al. (2009) did find a connection of UCN and preterm birth, stating that women giving birth before term did show lower UCN levels than women giving birth at or post term, Iavazzo, Tassis, et al. (2009) did not find a significant difference in UCN levels between the preterm birth and a control group. These are very recent studies, implicating the need of further prospective studies.

In another study by Torricelli, Voltolini, Biliotti, Giorlandino, De Pascalis, De Bonis, Mesuraca, Giovannelli, et al., (2009) the UCN levels in Down syndrome fetus pregnancies vs. normal pregnancies found to be at significantly lower levels in the trisomy group compared to the control group. It has to be noted though, that the study groups differed widely in number (115 controls, 15 cases) and that the use of non-parametric tests should have been preferred to analyze the data. As this was the only study on UCN in amniotic fluid and Down syndrome, it is obvious that further research will be needed to confirm or revise this observation.

2.3.2.3. Summary on UCN in human pregnancy

UCN plays an important role in human pregnancy and parturition, being related to uterine contractility and placental maintenance. Connections to pregnancy and birth complications are suggested, but a consensus on its important has yet to be reached and, at the present time, it is still poorly understood. UCN is relatively newly discovered peptide and additional studies are necessary to fully describe its role in human pregnancy.

Methodological issues in laboratory as well as statistics have to be addressed in future research.

2.3.3. Measuring CRH and UCN in amniotic fluid

As it is summarized in Table 2.2 and Table 2.3, wide ranges of UCN and CRH measured in amniotic fluid are reported. It has to be noted that earlier studies already pointed to the different findings in placental and maternal plasma UCN levels as well as to the variations of the placental and maternal CRH levels found in early studies (Clifton et al., 2000). The authors imply that the use of different assays and other variations in the methodologies lead to these results. This unusually wide range of levels might contribute to the controversial results and this issue has to be the focus of future studies.

Table 2.2. Studies on amniotic fluid CRH*, all concentrations transformed to pg/ml

Authors & Year	Assessment time	Assay	Kit	LOD	Level in pg/ml (*M* ± *SD*)
Florio et al., 2008	Not specified	RIA	Rabbit antirat CRF serum, synthetic human CRF NEN Life Science Products, Boston, Massachusetts, USA	2pg/ml	320 ± 40
Menon et al., 2008	At term	RIA	Rabbit Antiserum Peninsula Laboratories, Belmont, California, USA	2pMol/L	*Median* 251.22 *Range* 136.55 – 401-10
Salminen-Lappalainen & Laatikainen, 1990	15-17 weeks of gestation (WOG)	RIA	CRH-antiserum: synthetic human CRH conjugated with bovine thyroglobulin, rabit antiserum	4.9pg/tube (200 microliter)	44.25 ± 4.28
Stalla et al., 1989	Last trimester	RIA	Rabbit anti-serum Bachem Inc., Bubendorf, Switzerland	2pg/tube	120 ± 180
Torricelli, Voltolini, Galleri, et al., 2009	16.19 (*SD* 0.73) WOG	Quantitative colorimetric immunoassay	Phoenix Europe, Karlsruhe, Germany	.29ng/ml	1640 ± 680

Note. * If more than one group was assessed per study, levels of the groups with healthy pregnancy are reported. LOD = limit of detection; RIA = radioimmunoassay; CRF = corticotropin-releasing factor; CRH = corticotropin-releasing hormone; WOG = week of gestation.

Table 2.3. Studies on amniotic fluid UCN*, all concentrations transformed to pg/ml

Authors & Year	Assessment time	Assay	Kit	LOD	Level in pg/ml (*M* ± *SD*)
Iavazzo, Tassis, et al., 2009	15.9-23.7 WOG	Enzyme-linked immunoabsorbent assay (ELISA)	Human UCN EIA Phoenix Pharmaceuticals INC, Burlinghame, California, USA	0.2ng/ml	1600 ± 490
Torricelli, Voltolini, Biliotti, et al., 2009	15-16 WOG	Specific and sensitive immunoenzymatic assay	Phoenix Europe, Karlsruhe, Germany	.24ng/ml	900 ± 270
Torricelli, Voltolini, Galleri, et al., 2009	16.19 (*SD* 0.73) WOG	Specific and sensitive immunoenzymatic assay	Phoenix Europe, Karlsruhe, Germany	.24ng/ml	900 ± 260

Note. * If more than one group was assessed per study, levels of the groups with healthy pregnancy are reported. LOD = limit of detection; UCN = urocortin.

2.4. Consequences of being stressed during pregnancy

After the description of the general stress response and changes in the levels of CRH and UCN during pregnancy, general effects of stress during pregnancy will be described for pre-, peri- and postnatal complications.

2.4.1. Effects of stress on pregnancy and pregnancy complications

Stress during pregnancy has been related to a variety of pregnancy-associated complications (Roy-Matton, Moutquin, Brown, Carrier, & Bell, 2011). Especially pregnancy specific stress seems to lead to severe complications. This specific kind of stress might be caused by somatic symptoms (parallel with such changes in the body as: weight gain, preparation of lactation, maternal blood pressure and hepatic adaption), psychological concerns (health of the baby, the mother's own health, concerns about giving birth, about future parenthood and possible financial strains) or adjustment (relationship adjustment, adjustment to the bodily changes, adjustment to the future role as a parent[8]). Aside from studies on pregnancy complications in general, specific complications and outcomes have been evaluated, for example putting the experience of prenatal stress in relation to a higher risk of preeclampsia (Schneider et al., 2011), intrauterine growth retardation (Lesage et al., 2004), preterm and more difficult labor and delivery (Da Costa, Dritsa, & Larouche, 2000), higher risk for cesarean section (Martini, Knappe, Beesdo-Baum, Lieb, & Wittchen, 2010), low birth weight (Lee et al., 2011; Torche, 2011) and sadly also a higher risk for spontaneous abortion (Fenster et al., 1995; Neugebauer et al., 1996) and stillbirth (Wisborg, Barklin, Hedegaard, & Henriksen, 2008).

Another widely studied field in this context is preterm delivery, a complication with possible long-term health consequences (Pike, 2005) such as higher morbidity for the newborn (Buske-Kirschbaum et al., 2007). Various research groups have found a connection between higher maternal stress and preterm birth (Austin & Leader, 2000; Copper et al., 1996; Dunkel-Schetter, 1998; Hogue, Hoffman, & Hatch, 2001; Lobel, Cannella, Graham, et al., 2008). In this context, the relationship of high prenatal stress and low birth weight (Dunkel-Schetter,

[8] For further reading about pregnancy specific stress, see e.g. Adamczak & Wolf, 2010; Affonso, Liu-Chiang, & Mayberry, 1999; Burst, 1987; Bustamante, Copple, Soares, & Dai, 2010; Fox, Bruce, & Combs-Orme, 2000; Hart & McMahon, 2006; Johnson, Burrows, & Williamson, 2004; Lobel, Cannella, Graham, et al., 2008; Park, Moore, Turner, & Adler, 1997; Stark, 1997; Statham, Green, & Kafetsios, 1997; Wallace & Gotlib, 1990; Yali & Lobel, 1999, 2002.

2011) is not surprising.

An interesting evolutionary theory on preterm birth in connection with stress states that preterm birth actually might be a reaction or global maternal adaptation to poor or stressful conditions and therefore to insufficient internal environments for the fetus (Pike, 2005). A maternal adaptation in this case, with goal of reducing the costs of a single pregnancy low (Pike, 2005). While first several fetal responses (i.e. accelerated maturation) take place, an unchanged stressful intrauterine environment starts a feed forward mechanism – a hormonal cascade that eventually leads to preterm birth (Pike, 2005).

Interestingly, pregnancy related stress was also found to be connected with unhealthy behavior (such as smoking, caffeine intake, malnutrition) during pregnancy (Lobel, Cannella, Graham, et al., 2008). This fact has to be taken into account in future studies, as this might mediate the direct effects of prenatal stress and complications and outcomes.

2.4.2. Effects of prenatal stress on the well-being of mother and child after birth

The impacts of prenatal stress do not stop at parturition. Perceived maternal prenatal stress may predict the cortisol stress reactivity of the newborns not only shortly after birth (Leung et al., 2010) and in the first year of life (Tollenaar, Beijers, Jansen, Riksen-Walraven, & De Weerth, 2011), but possibly throughout the entire lifetime. Infants exposed to higher maternal psychosocial prenatal stress and therefore higher maternal cortisol levels show higher cortisol responses to stressors and a slower behavioral recovery after being stressed (Poggi Davis, Glynn, Waffarn, & Sandman, 2011). In ten year old children of high anxiety-mothers (anxiety used as an indicator of chronic stress), there was a significant correlation of maternal anxiety and the offspring cortisol awakening response.(O'Connor et al., 2005) The few human studies on this subject that have been carried out so far (which are moreover mostly retrospective) are backed up by various animal studies that show an abnormal HPA axis regulation and a lack of adaptation of the HPA and impaired coping in adult offspring in rodents as well as non-human primates (Weinstock, 1997). Moreover, children of mothers with high perceived prenatal stress suffer more from attention deficit disorder (ADHD), conduct disorder and separation anxiety disorder in their early childhood until the age of ten (Martini et al., 2010).

Stress is a known risk factor for affective disorders including depression and several anxiety

disorders (Green et al., 2011; Pawluski, van den Hove, Rayen, Prickaerts, & Steinbusch, 2011), schizophrenia and other psychotic disorders (Markham & Koenig, 2011). Prenatal stress has also been shown to be a risk factor for psychopathology in general (Huizink, Mulder, & Buitelaar, 2004) as well during pregnancy as postpartum. Women suffering from postpartum depression retrospectively report more stressful life events and therefore more perceived stress during pregnancy than women without this affective disorder after birth (O'Hara, 1986). More specifically, women with a higher cortisol and psychological reactivity during psychosocial stress show higher levels of postpartum depressive mood (Nierop, Bratsikas, Zimmermann, & Ehlert, 2006). It has been suggested that there is a connection between prenatal stress and the vulnerability to depression, anxiety and probably other neuropsychiatric disorders as well (Miyagawa, Tsuji, Fujimori, Saito, & Takeda, 2011). In mice, excessive prenatal stress resulted in anxiety behaviors together with an alteration of the serotonergic system related to depression, namely a disruption in the development of 5HT-neurons (Miyagawa et al., 2011). In a rat study, prenatal stress induced adult vulnerability to stress, also showing up as heightened anxiety- or fear related behaviors, physiologically correlated with alterations in the noradrenergic system and the HPA activity (Green et al., 2011). Changed HPA activity is a common finding in animal studies on prenatal stress; this has also been brought in connection with consequently impaired coping with stressors (Weinstock, 1997).

2.4.3. Possible pathways – The fetal programming hypothesis

The exact pathways by which prenatal stress affects pregnancy, the mother and the unborn, still remain unclear. Nevertheless, some theories have gained general acceptance by being backed by various empirical studies. The generally accepted hypothesis of possible pathways of the impact of prenatal stress on the wellbeing, development and health of the newborn is fetal programming of the HPA (Charil et al., 2010; Egliston, McMahon, & Austin, 2007; Van den Bergh, Mulder, Mennes, & Glover, 2005; Welberg, Seckl, & Holmes, 2001). One of these programming effects might be the alteration of the negative feedback mechanism (Maccari, Darnaudery, & Morley-Fletcher, 2003; Markham & Koenig, 2011), resulting in prolonged glucocorticoid secretion and at the same time a reduction of central glucocorticoid (Maccari et al., 2003) and mineralocorticoid receptors (Tamura, Sajo, Kakita, Matsuki, & Koyama, 2011). A connection with increased CRH levels is also being discussed (Markham & Koenig, 2011). Newer reviews (Markham & Koenig, 2011) also suggest a role of 11- beta-hydroxisteroid

dehydrogenase (11-beta-HSD), an enzyme also present in the amniotic fluid. This enzyme exerts a protective function as it transforms active cortisone into inactive cortisol, preventing the fetus from being exposed to even higher maternal glucocorticoid levels (Welberg, Thrivikraman, & Plotsky, 2005).

This early imprinting may have permanently altering effects on the offspring such as altered endocrine system regulations with resultant health consequences (Kapoor, Dunn, & Kostaki, 2006). Prenatal stress may negatively influence fetal brain development (including areas such as the hypothalamus, hippocampus, amygdala, corpus callosum, anterior commissure, cerebral cortex and cerebellum) through effects mediated by maternal and fetal HPA hormones (Charil et al., 2010). The effects of prenatal stress via alterations in brain development (Kinsella & Monk, 2009) include problems in regulating behavior and emotions (Egliston et al., 2007) and cognitive and emotional problems such as increased risk of attention deficits or hyperactivity, anxiety and learning problems demonstrable, for instance, in the delay of language development (Talge, Neal, Glover, & Early Stress Translational Research an Prevention Science Network: Fetal an Neonatal Experience on Child and Adolescent Mental Health, 2007).

In this context, an evolutionary perspective may help explain the pathways, even though nowadays the cost of these alterations outweigh the evolutionary benefits: A hyperreactive HPA – including extra vigilance and attention – might have been a survival enhancing adaption to a stressful environment during evolution (Talge et al., 2007).

It has to be noted, that some newer studies do find differences in effects considering the time of exposure to prenatal stress, and in consequence, altered glucocorticoid levels. While high stress levels in early pregnancy resulted in lower scores on an infant development scale at the age of 12 months, higher maternal cortisol levels in late pregnancy were associated with an accelerated development of the infant in the first life year (Davis & Sandman, 2010). Other research groups suggest the model of a double-edged sword, suggesting that mild stress promotes brain functions while severe stress might impair neuronal function in a short and long term perspective (Avishai-Eliner, Brunson, & Sandman, 2002), this theory might also be seen in the above mentioned evolutionary thought ’function’ of preterm birth (Pike, 2005).

The sensitivity to timing might be related to the different stages of fetal brain development

and therefore also different levels of vulnerability. Other factors that modulate the effects of prenatal stress are intensity and duration of the stressor, as well as the gender of the offspring (Weinstock, 2008).

More recently, the theory of prenatal programming has been more specifically discussed, with talk of "prenatal programming of postnatal plasticity", as, for instance, in the article with this very title by (Pluess & Belsky, 2011). In this newer way of thinking, nature and nurture come together when it comes to developmental plasticity, with both being thought to play a role in evolutionary adaptation.

2.4.5. A specific pregnancy related stressor – perceived stress due to prenatal diagnostics

As depicted above, all kinds of stress have an influence on the course and outcome of human pregnancy. Specific pregnancy related stress, however, seems to have an even higher impact than general stress. Apart from somatic symptoms, bodily changes and adjustments to the already mentioned concerns about the future parenthood – as well as concerns about the health of the baby – are stressful for the pregnant women. These stressors are highly individual and seldom comparable – for our research, some sort of a standardized stressor was needed, a stress factor that causes some sort of stress in every woman encountering it.

In connection with concerns about fetal health, a new field has opened and expanded over the last years and decades: prenatal diagnosis. Prenatal diagnosis offers opportunities to the future mothers, but can also cause stress and be a burden. Amniocentesis, utilizing a transabdominal needle - with the aim of gathering fetal cells from the amniotic fluid, is one possibility for prenatal diagnostics. It is an invasive procedure, bearing an average risk of 0.74% of complications – possibly leading to the loss of the child (Bettelheim, Kolinek, Schaller, & Bernaschek, 2002). Access to genetic information of the fetus is gained by puncture of the amnion and obtaining as sample of amniotic fluid. All invasive procedures are painful, although the perceived amount of pain is reported to be tolerable by most women (Csaba, Bush, & Saphier, 2006; Harris et al., 2004), with amniocentesis judged as less painful than transabdominal chorionic villus sampling but more painful than transcervical chorionic villus sampling. Moreover, the expected pain before the procedure is significantly higher than the effectively perceived pain during the sampling. Increased perceived pain has been shown to be correlated with higher maternal anxiety (moderate to high correlation: Ferber, Onyeije, Zelop,

O'Reilly-Green, & Divon, 2002; low correlation: Harris et al., 2004), and maternal anxiety was also associated with increased affective dimensions of pain (Harris et al., 2004). Whether previous experience with invasive prenatal procedures is associated with lower (Ferber et al., 2002) or higher (Harris et al., 2004) perceived pain is still a matter of controversy.

As it is the case with other prenatal screenings, it has been shown that amniocentesis may be a stressful, anxiety promoting event for pregnant women (Brajenović-Milić, Martinac Dorcić, Kuljanić, & Petrović, 2010; Cederholm, Sjödén, & Axelsson, 2001; Kowalcek, Mühlhoff, Bachmann, & Gembruch, 2002; Leithner et al., 2004; Marteau et al., 1992; Ng, Lai, & Yeo, 2004; Sarkar, Bergman, Fisk, & Glover, 2006). It has been shown that about one third of the women experience these invasive procedures as high impacting major strains that are associated with high levels of distress and anxiety (Cederholm et al., 2001). State anxiety levels during the procedures are, moreover, significantly positively correlated with maternal plasma cortisol, even when gestational age and time of collection were controlled for (Sarkar et al., 2006). The stress women suffer in confrontation with amniocentesis has been shown to be not only due to the procedure itself, but also due to the possibility and the fear of negative results of the screening (Marteau et al., 1992).

2.4.6. Prenatal stress – is there an antidote?

Protective factors and possible interventions have also been studied and affirmed. Psychosocial resources such as self-efficacy and daily uplifts have been shown to dampen the psychophysiological stress response and go along with higher mood levels (Nierop, Wirtz, Bratsikas, Zimmermann, & Ehlert, 2008). Stronger personal resources are also related with higher birth weights of the babies and lower reported stress levels (Rini, Dunkel-Schetter, Wadhwa, & Sandman, 1999). High stress women profit from a special group program on prenatal care, that results in decreased reported stress levels during pregnancy and lower depression rates postpartum than is the case with women not in this program – and in general women in the program show improved psychosocial outcomes (Ickovics et al., 2011). Relaxation techniques such as progressive muscle relaxation and guided imagery decrease psychological stress experience and endocrine stress responses (Urech et al., 2010). Stress management programs result in lower reported stress levels, as well as lower depressive or generally negative affect experienced in pregnancy as well as lower morning cortisol levels compared to women without this kind of intervention (Urizar et al., 2004).

Further studies are necessary to better understand the pathways that make these improvements possible and to develop prevention campaigns and programs for pregnant women at risk for high stress.

2.4.7. Summary on prenatal stress

A wide array of possible negative impacts of pre-natal stress on the course of pregnancy, birth, infant development and wellbeing of the mothers after birth has been shown. It clearly shows that the impacts may be severe and may have long-term consequences and are therefore not at all negligible. Specific pregnancy related stress is not a phenomenon of (high) risk pregnancies only; it is experienced in any pregnancy. A recent study has shown that healthy low risk women experienced pregnancy related stress as well (Lynn, Alderdice, Crealey, & McElnay, 2011). The authors moreover point out that the current medicinal system is not equipped to identify highly stressed women, even though the severe consequences of prenatal stress are known (Lynn et al., 2011).

Despite all the studies depicted above, it has to be noted that the pathways are still a matter of research and that there are limitations to the human studies conducted up to the present time. Often, generalization is limited due to the specificity of samples and due to very heterogeneous definitions and measurements of stress, as a recent review shows (Beydoun & Saftlas, 2008). The authors suggest intervention and population-based studies with large samples and repeated, multidimensional and standardized stress assessments including biomarkers. Other reviews support this, inasmuch as reported stress and biomarkers seem not straightforwardly related in pregnancy (Harville et al., 2009). When the postpartum consequences are studied, more attention should also be paid to gender differences, as animal studies have shown different effects of stress-induced heightened corticosterone levels on brain and endocrine systems between male and female offspring (Weinstock, 2007).

2.5. General summary

The above-depicted sections show the important roles that psychological and physiological stress, some of its biological correlates (namely UCN and CRH), stress-related mental consequences such as depressive mood and anxiety and coping with stressors play in human pregnancy. Disturbances in the regulation or overloads in any of these mechanisms may have severe and long lasting impacts on pregnancy, pregnancy outcomes, the (unborn) baby and/or

the mother. While some of these factors have been well explored, the pathways still remain only partly understood. Acute perceived stress and coping with acute stressful situations have rarely been studied. CRH and its relatives have been suggested as possible mediators of these mechanisms, but their double role in human stress reactions and human pregnancy is still poorly understood. This thesis aims to contribute to a better understanding of perceived stress during pregnancy as well as to methods of psychophysiological coping with perceived stress and to their possible consequences on psychological wellbeing.

3. Psychological coping during pregnancy

The consequences of psychosocial factors such as stress, anxiety and depression point to the importance of changing perspectives and looking at how pregnant women actually deal with such adversities – their psychosocial adaptation or their coping styles. After a short look at the general meaning of coping and a digression into a discourse on different coping measures especially during pregnancy; the impacts of coping during pregnancy will be discussed.

3.1. Psychological coping - a widely and controversially discussed concept

In this section, the psychological manner of coping is described, focusing on coping during pregnancy. First, the basics of coping and the operationalization of coping according to Lazarus and Folkman (Folkman & Lazarus, 1980; Folkman, Lazarus, Gruen, & DeLongis, 1986; Lazarus & Folkman, 1987) will be discussed.

According to the definition of Folkman and Lazarus, coping means the manners by which people manage stressful situations, including behavior, cognitions and emotions (Folkman & Moskowitz, 2004). In the center of their definition is the cognitive appraisal of a situation, divided into the so called primary appraisal, during which the individual threat of a situation is assessed, and the secondary appraisal, meaning the evaluation of the personal resources to handle the situation (Da Costa, 1997). Already in early studies, correlations of coping styles with psychological symptoms as well as with current health status were discovered (Folkman et al., 1986), building the base of a new field of research, not only looking at the effects of stress,but considering the importance of the way people manage it.

Based on the theories of Lazarus and Folkman, Da Costa (1997) elucidates three main types of coping: Two of them have been traditionally described, namely problem- and emotion-focused coping, while the third one, avoidance-focused coping is a more recent concept. While orientation on the problem means focusing on possible solutions and managing the problem, emotion-orientation comprises all actions directed at lowering emotional-distress. Avoidance-orientation includes all strategies aimed at denying or minimizing the actual situation and attempts at avoiding being reminded of it.

It is still widely propagated that coping is a stable trait, but Lazarus and Folkman already insisted a good while ago that coping is significantly influenced by the situational context and the individual appraisal of a situation and that therefore single coping assessments are not considered to be reliable for a person's coping style (Lazarus & Folkman, 1987). Given the impact of context in specific situations, it is therefore reasonable to examine enduring conditions such as pregnancy specifically.

The common idea that problem-focused coping is mostly used in situations with personal control over the outcome and less in non-controllable situations, and, moreover, is harmful in the later situations might be oversimplified, considering controllable aspects of superficially uncontrollable situations (Folkman & Moskowitz, 2000). The authors even found increase of positive mood when problem-focused coping was used in mostly uncontrollable situations such as care giving in terminal illnesses. This reveals that the assignment of certain coping styles to certain types of situations and challenges is complex and may vary depending on the individuals involved and influenced by their character traits.

The importance of healthy and sufficient coping skills has also led to the endeavor to identify factors influencing the way expectant mothers manage stressful events. Especially sociodemographic factors such as age, income and education have been shown to be strongly related to coping styles during pregnancy (Borcherding, 2009; Yali & Lobel, 1999).

3.2. Impact of coping during pregnancy

Coping during pregnancy has been examined in high risk (Yali & Lobel, 1999), varying medical risk (Hamilton & Lobel, 2008) as well as in healthy pregnancies (Borcherding, 2009; Huizink, Robles de Medina, Mulder, Visser, & Buitelaar, 2002a). An overview of these studies is given in Table 3.1. While some coping styles such as positive appraisal (Yali & Lobel, 1999, 2002) are related to less distress during pregnancy, poor coping skills are related to negative pregnancy outcomes, i.e. low birth weight (Borders, Grobman, Amsden, & Holl, 2007) and reduced emotional well-being, specifically depressive mood during pregnancy (Da Costa, Larouche, Dritsa, & Brender, 2000). It is suggested that the effects of coping on distress are mostly direct, except for early pregnancy, where emotion-focused coping may be a mediating factor between stressor and response (Huizink, 2000).

Table 3.1. Examples of current research on coping during pregnancy

Author(s) and year of publication	Title of the study	Coping assessment	Sample size and point in time	Main results
Normal pregnancy				
Borcherding, 2009	Coping in healthy primigravidae pregnant women	PCI[1], CISS[2]	n = 159 3rd trimester	Most women use prayer and task coping in the third trimester More frequent use of preparation and distraction associated strategies is associated with younger age More frequent use of prayer, task and distraction associated coping strategies is associated with non-white race. More frequent use of preparation strategies is associated with lower educational level
Huizink et al., 2002a	Coping in normal pregnancy	UCL-19[1]	n = 231 across all 3 trimesters	Small temporal variations in coping Most women use emotion-focused coping during early pregnancy Emotion focused coping is associated with high education and low internal locus of control Negative correlation of emotion-focused coping with pregnancy complaints Positive correlation of problem-focused coping with pregnancy complaints Factor structure of the UCL-19

Mixed risk pregnancies				
Hamilton & Lobel, 2008	Types, patterns, and predictors of coping with stress during pregnancy: Examination of the Revised Prenatal Coping Inventory in a diverse sample	NuPCI[4]	n = 321 Early, mid- and late pregnancy	Most frequent use of spiritual coping throughout pregnancy, least often use of avoidance coping Planning was used more consistently throughout pregnancy, spiritual and avoidance coping differed Planning is associated with high optimism and pregnancy-specific distress Avoidance is associated with high state anxiety and pregnancy specific distress Spiritual coping is associated with religiosity and optimism Coping responds to the demands across pregnancy
Yali & Lobel, 2002	Stress-resistance resources and coping in pregnancy	A revised version of the PCI[1]	n = 163 Early and mid-pregnancy	Less frequent use of avoidance and lower emotional distress is associated with greater social support More frequent use of avoidance and preparation coping and higher levels of emotional distress are associated with greater social support Positive appraisal is the only coping strategy associated with less distress Coping is not associated with distress over time; it is only associated with resources and distress in early pregnancy

High medical risk pregnancies				
Yali & Lobel, 1999	Coping and distress in pregnancy: an investigation of medically high risk women	PCI[1]	n = 167 midpregnancy	More frequent use of coping strategies such as avoidance, preparation for motherhood, and substance abuse is associated with greater pregnancy specific distress More frequent use of positive appraisal as a coping strategy is associated with less pregnancy specific distress The associations of distress and ways of coping differ, when levels of global, non-specific stress is controlled in the statistical analysis.

Note. [1]PCI: Prenatal Coping Inventory. Subscales (Borcherding, 2009) include preparation (planning for the arrival of the baby, talking about others about having a baby; 8 items), positive appraisal (focus on rediscovering important things in life, thinking about pregnancy-related experiences, 5 items), prayer (2 items) and avoidance (sleeping, overeating, social withdraw, taking out frustrations on other people; 7 items).

[2]UCL-19: Utrecht Coping List. Short-form of the Utrecht Coping List 30. Factor-analytic based subscales (Huizink et al., 2002a): Emotion-focused coping (show your emotions, seek comfort and understanding, show your concern) and problem-focused coping (look at the problem from different viewpoints, think of several solutions, work goal-oriented to solve problem.

[3]CISS: Coping Inventory for Stressful Situations. Subscales (Borcherding, 2009) include problem-focused/task coping (efforts to solve problems, alter the stressful situation), emotion-focused coping (self-centering, perceiving negative emotions that may even worsen the stressful situation) and avoidance coping (use of diversions and distractions).

[3]NuPCI: Revised Prenatal Coping Inventory. Factor analysis based subscales (Hamilton & Lobel, 2008) include planning-preparation coping, avoidance coping and spiritual-positive coping.

While assessment tools vary and research groups do not agree on the ideal tool for the examination during pregnancy, authors agree on the fact that pregnant women use a (wide) variety of coping styles and that coping might be influenced by psychosocial or sociodemographic factors (Borcherding, 2009; Hamilton & Lobel, 2008). Only a few studies bring coping and physical pregnancy complications such the outcome of preterm labor (Demyttenaere, Maes, Nijs, Odendael, & Van Assche, 1995) directly together. In their early study, the authors found that in the highly stressful situation of preterm onset of labor, it is protective to use palliative and social support seeking coping strategies, while active coping did have a negative impact on the outcome. As mentioned before, however, the focus of previous studies mostly lay in coping and its consequences for well-being and mood of the pregnant women.

Specific findings affirmed by several research groups addressed coping during pregnancy and depressive mood or depression during pregnancy and after birth. In 1995, Demyttenaere, Lenaerts, Nijs, & Van Assche suggested a predictive value of the individual coping style for depression during the third trimester and six months, but not five days and six weeks postpartum. A higher use of depressive coping was especially predictive for higher levels of depression six months after having given birth. Later studies found that especially higher use of emotion oriented coping strategies was associated with a higher frequency of depressive moods during pregnancy (Da Costa et al., 2000; Lobel, Hamilton, & Canella, 2008).

3.3. Different coping measures used during pregnancy

It has been stated that the measurement of variables such as coping might be difficult during pregnancy (Ayers, 2001), as it is difficult to differentiate between general and pregnancy specific coping strategies.. In the current literature, a wide variety of coping inventories are used to assess maternal prenatal coping styles (Dunkel-Schetter, 2011), as has been illustrated in Table 3.1. This reveals how difficult it might be when it comes to a comparability of studies in this context, an issue that has already been addressed elsewhere (Lobel, Hamilton, & Canella, 2008). In the following, the mainly used or revised assessment tools will be listed (with sample studies in brackets):

- The Coping Inventory for Stressful Situations (CISS) (e.g. Borcherding, 2009),
- the Prenatal Coping Inventory (PCI) (e.g. Yali & Lobel, 1999) or parts of the PCI (e.g. Borcherding, 2009),

- the Revised Prenatal Coping Inventory (NuPCI) (e.g. Hamilton & Lobel, 2008),
- the Utrecht Coping List (UCL-19) (e.g. Huizink et al., 2002a),
- the Coping Responses Inventory (CRI) (e.g. Ayers, 2001) and
- the Ways of Coping Questionnaire (WCQ) (e.g. Ayers, 2001).

While some assessments UCL-19, CISS, CRI and WCQ are general coping questionnaires; the PCI and NuPCI are questionnaires for pregnancy-specific coping. The authors of the latter instruments insist that general coping scales are not sufficient for the assessment of coping under the special circumstances of pregnancy (Huizink et al. 2002b; Yali & Lobel, 1999). On the other hand, to compare pregnant with non-pregnant women, tools must be used that are applicable to both samples. It is, however, probable that usual coping questionnaires are not able to fully capture coping strategies used during pregnancy. Therefore the development of a new assessment tool would be recommended. To capture both pregnancy-related and general coping styles it might be helpful for future studies to apply both specific and general instruments – as for instance was done by Borcherding (2009). Furthermore, a study comparing the validity of both assessment styles may be necessary.

Aside from the variety of assessment tools, even more difficulties are caused by the fact that the different assessment tools use different, often non-comparable subscales, sometimes similarly named but not covering the same concepts (see also Table 3.1, i.e. planning/preparation). This can lead to confusion and aggravates the already existing difficulty of comparing current research on coping during pregnancy.

3.4. Summary on coping during pregnancy

It has been shown that the assessment of coping during pregnancy is a complex, still widely and hotly debated field. The ’gold standard’ considering assessment time points and assessment tools has thus far not been established. Both pregnancy specific and general coping measures have their advantages and disadvantages when taking into account the main aims of the studies in which they are applied. At least there is some consensus about the necessity of examining coping during pregnancy across time, as the findings across the three trimesters are very heterogeneous (Lobel, Hamilton, & Canella, 2008). A recent review highlights the fact that during pregnancy, a general attribution of *good* or *bad* to certain coping styles is impossible, as it seems to be highly dependent on personal characteristics, environment and context (Lobel, Hamilton, & Canella, 2008).

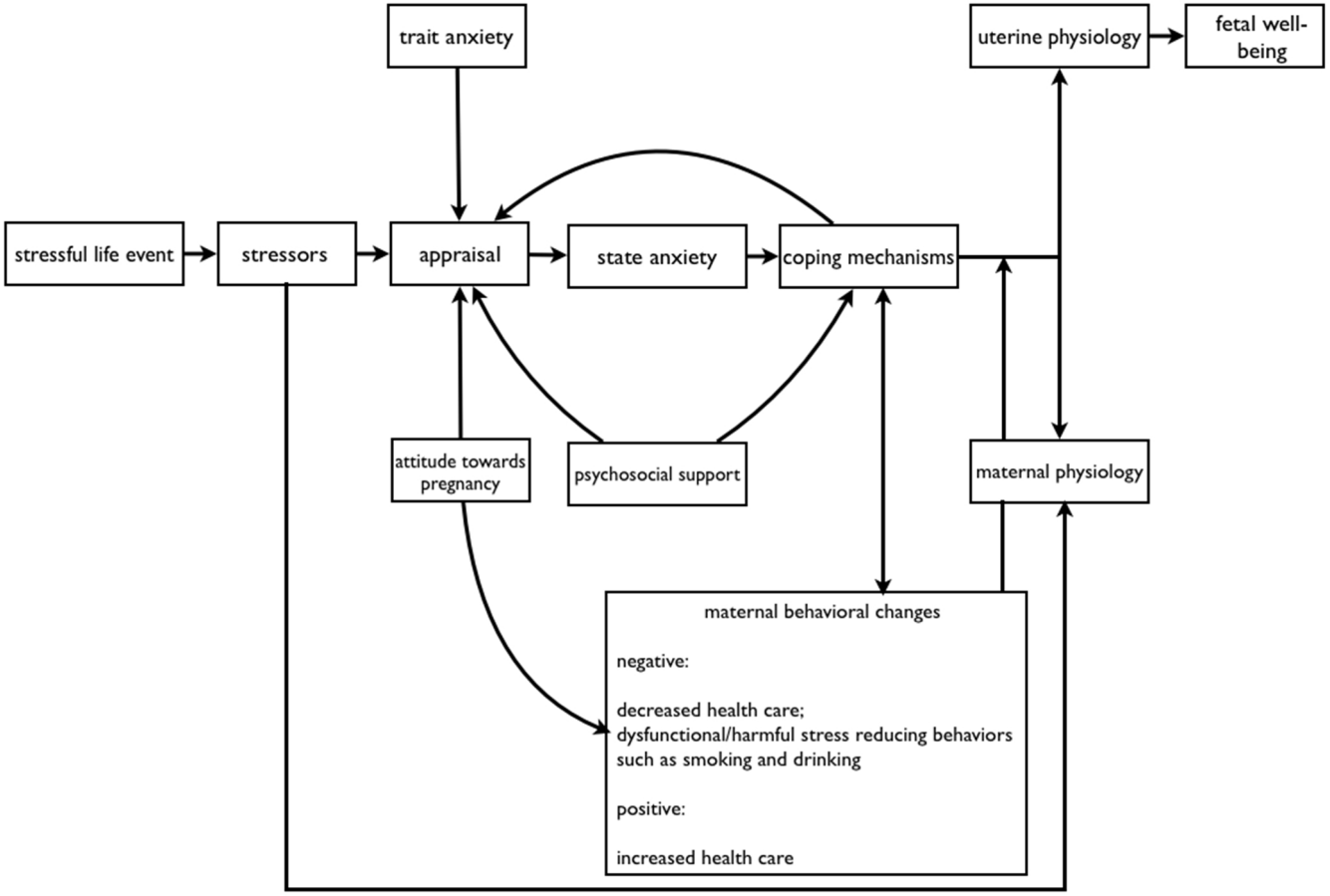
4. Anxiety during pregnancy

In this chapter, the concepts of general and pregnancy specific anxiety during pregnancy and their influence on the course and outcome of pregnancy as well as the well-being of mother and child after birth will be described. First, it is important to see that anxiety, stress, depression and their impacts are interrelated.

4.1. The interplay of anxiety, stress, depression and coping

Anxiety is interwoven with the concepts of stress, mood and coping, not only during pregnancy (Reading, 1983). The close connections among these concepts can be seen in Figure 4.1.The majority of the studies actually do assess anxiety and stress simultaneously, and sometimes mix both concepts or use anxiety as an indicator for chronic stress (as for instance in O'Connor et al. (2005). It has been suggested that maternal anxiety might be a moderating factor to the impact of stress on pregnancy (Reading, 1983), prenatal stress on the other hand has been shown as a moderator or mediator when it comes to the transmission of anxiety to the offspring (Martini et al., 2010). It has also been shown that depressed pregnant women showed significantly higher anxiety levels than non-depressed women (Parcells, 2010). However, a close connection among anxiety, stress and depression when it comes to (negative) influences on pregnancy, birth and birth outcome has to be assumed and therefore, a closer look at anxiety and its possible consequences during pregnancy is necessary.

Figure 4.1. The influences of maternal anxiety, stress and coping on pregnancy, adapted with permission[9] (originally by Reading, 1983)



Note. The interaction of stress, anxiety and coping and its effects on human pregnancy.

[9] No formal permission was required due to the APA guidelines for the use of copyrighted content (fair use).

4.2. Anxiety and its influences during the course of pregnancy

During pregnancy, apart from general trait and state anxiety, specific pregnancy related anxiety including birth anxiety, worries about the child's health and about the one's appearance has to be regarded as a unique syndrome (Huizink, Mulder, Robles de Medina, Visser, & Buitelaar, 2004), also in healthy pregnancies. It seems that general state anxiety is higher in the third – compared to the first and the second trimester (Da Costa, Larouche, Dritsa, & Brender, 1999; van Bussel, Spitz, & Demyttenaere, 2009) and was highly to moderately correlated to frustrations and pregnancy specific stress throughout the course of pregnancy (Da Costa et al., 1999). Bhagwanani, Seagraves, Dierker and Lax (1997) found lowest levels of general anxiety at 22 to 26 weeks of pregnancy. More frequent use of depressive coping and higher neuroticism seem to be predictors for both general and pregnancy specific anxiety (van Bussel et al., 2009).

Birth anxiety is present in nulliparous and parous pregnant women (Fenwick, Gamble, Nathan, Bayes, & Hauck, 2009; Laursen, Hedegaard, & Johansen, 2008; Nilsson, Bondas, & Lundgren, 2010). Higher birth anxiety in nulliparous women compared to parous women (Fenwick et al., 2009) was found. Still, birth anxiety of parous women depends on the feelings they relate to their previous birth experience, as it can also be associated with suffering and even trauma (Nilsson et al., 2010).

In a Danish study, the prevalence of birth anxiety is reported to lie between 7.4-7.5%. Interestingly, Laursen, et al. (2008) found equal levels of birth anxiety in early and late pregnancy, but discovered that about 50% of the women fearful at the beginning of their pregnancy have no fear in the late stage, counterbalanced by an equal number of women who started without fear and developed fear before the third trimester. Only 3.2% of their sample expressed birth anxiety at both assessment time points. During the last trimester itself, birth anxiety seems to be stable (Sieber, Germann, Barbir, & Ehlert, 2006). It has to be noted, that there are also studies that report an increase in pregnancy specific anxiety, at a frequency similar to general anxiety across pregnancy (van Bussel et al., 2009).

Apart from previous childbirth experience, various sociodemographic and personal factors have been studied and brought into a connection with birth anxiety and anxiety during preg-

nancy. In their extensive Danish National Birth Cohort study (sample size = 30,480), Laursen et al. (2008) found that a minimal educational background and unemployment, a deficient social network, young age, smoking and low self-rated health were connected to birth anxiety. There are also significant positive correlations with phobic anxiety, well-being of the mother and the baby, obsessive-compulsive behavior and a negative correlation with the partner's desirability of the pregnancy (Sieber et al., 2006). Psychiatric disorders such as previous anxiety and/or depression and severe life events such as abuse are also highly predictive for higher birth anxiety. Sieber et al (2006) found a negative correlation of birth anxiety and self-efficacy of pregnant women. This finding is also interesting in connection of another study, that found higher requests for cesarean sections in women with high birth anxiety, a decision that changed in 86% of the cases after a special intervention program for the fearful women (Nerum, Halvorsen, Sorlie, & Oian, 2006).

There seem to be ethnic differences as well, as it could be shown that Australian women were more fearful concerning giving birth than Swedish women (Fenwick et al., 2009); more widespread investigations of cultural/ethnic differences are still necessary.

4.3. The impacts of prenatal anxiety on the course of pregnancy and delivery

Anxiety is also associated with pregnancy complications such as higher risks for preeclampsia (Kurki, Hiilesmaa, Raitasalo, Mattila, & Ylikorkala, 2000), fetal intrauterine growth retardation (Gawlik et al., 2010) or preterm birth (Dole et al., 2003; Gawlik et al., 2010; Orr, Reiter, Blazer, & James, 2007). Mancuso et al. (2004) moreover found a close association among high pregnancy specific anxiety, high maternal blood CRH levels and preterm delivery. It has to be noted that there are differing results for general anxiety (Dayan, Creveuil, & Marks, 2006) and anxiety disorders (Berle et al., 2005), where no connection to preterm delivery can be found.

Anxiety, so as stress and depression, can have impacts on fetal heart rate, fetal activity and (consequently) fetal neurobehavioral development (Kinsella & Monk, 2009). Changes in fetal heart rate (FHR) during a stress test undergone by the mother (Stroop task) were significantly and positive correlated to the maternal trait anxiety score, while the changes in FHR were not related to the maternal physiological activity, nor was the maternal physiological reaction to

the task related to their anxiety scores (Monk, Myers, Sloan, Ellman, & Fifer, 2003). An older study of the same research group already showed significant increases in FHR in the group of pregnant women with high maternal state anxiety compared to the group with below average state anxiety (Monk et al., 2000).

4.4. The impacts of prenatal anxiety on the well-being of mother and child after birth

Recently, pregnancy specific anxiety, such as the fear of giving birth to a handicapped child, was shown to be a predictor of the babies' cortisol reactivity to a stressor after birth, with higher maternal anxiety going along with higher stress reactivity of the baby (Tollenaar et al., 2011). This study is backed by previous research showing that higher prenatal maternal anxiety is related to altered HPA-activity of pre-adolescent children: specifically to elevated awakening and afternoon cortisol levels (O'Connor et al., 2005), and attenuated day cortisol profile, namely high flattened profiles, in adolescence (Van den Bergh, Van Calster, Smits, Van Huffel, & Lagae, 2008).

Additionally, altered brain development has been shown to be associated with prenatal maternal anxiety. Volume reductions in gray matter in several areas of the brain such as the prefrontal cortex, the premotor cortex, parts of the temporal lobe, the postcentral gyrus and the cerebellum in children have been related to antenatal maternal anxiety (as early as 19 weeks of gestation) independently of possible postnatal stress (Buss, Davis, Muftuler, Head, & Sandman, 2010). A brain-related fetal programming hypothesis has been conceived in connection with mixed-handedness, a phenomenon that seems to be associated with higher maternal prenatal anxiety (Glover, O'Connor, Heron, Golding, & The ALSPAC Study Team, 2004) and, as well, to neurodevelopmental problems including autism, ADHD and dyslexia (Van den Bergh et al., 2005).

Moreover, antenatal maternal anxiety can have long-term effects on the child's psychological development, including behavioral and emotional disturbances (Glover & O'Connor, 2002). Correlations have been maternal anxiety conditions and childhood problem behavior (Loomans et al., 2011) as well as emotional and behavioral problems (conduct problems, emotional problems, hyperactivity/inattention) in early (O'Connor, Heron, & Glover, 2002;

O'Connor, Heron, Golding, Beveridge, & Glover, 2002) and middle (O'Connor, Heron, Golding, Glover, & The ALSPAC Study Team, 2003) childhood.

Interestingly, the effect of maternal anxiety on problem behavior seems to be higher in boys, as the increase in problematic behavior was shown to be higher compared to girls (Loomans et al., 2011). Altered HPA-function in relation to antenatal maternal anxiety indeed was associated with depressive symptoms in female adolescents, but not in male (Van den Bergh et al., 2008). Both findings suggest gender differences in the effects of prenatal stress, although the pathways remain unclear.

Considering the new mothers, birth anxiety was shown to be predictive for unfavorable psychological status postpartum (Sieber et al., 2006), while diagnosed anxiety disorders, such as generalized anxiety disorder and social phobia, may predict postnatal depression (Coelho, Murray, Royal-Lawson, & Cooper, 2011).

4.5. Summary on prenatal anxiety

Prenatal anxiety, birth anxiety as well as general anxiety or clinically diagnosed anxiety disorders have been shown to be connected with pregnancy and birth complications and influencing the well-being of the child after birth. These relations are not only seen in connection with prenatal stress (moderating/mediating hypothesis), but also independently.

It has to be noted that due to a high variability of assessment instruments, operationalizations of anxiety and different choices of confounding variables the analysis were controlled for (if ever), current research still lacks comparability and future studies should address this issue. Inasmuch as birth anxiety should be regarded as a distinctive syndrome (Huizink, Mulder, Robles de Medina, et al., 2004), both birth anxiety and general anxiety were taken into account in our experimental study design.

5. Depressive mood during pregnancy

Depression is a major psychiatric disorder that has a higher prevalence among women than among men and affects women especially in their reproductive years (Parcells, 2010). As is the case with stress and anxiety, depressive mood and depression during pregnancy are related to pregnancy complications and consequences for mother and child. Earlier studies focused on postpartum depression and its consequences on the mother-child bonding and the development of the infant. Later studies discovered that depression or depressive symptoms during pregnancy are at found at least as often as such conditions are after birth (Da Costa, Larouche, et al., 2000) and that the consequences of this psychological distress is similar to those of stress and anxiety. It seems that pre-and postpartum depression are structurally and symptomatically different, with prenatal depression resembling melancholic depression (heightened cortisol levels) and postpartum depression's being equivalent to atypical depressions with low cortisol levels or mild versions of bipolar II depressions (Kammerer, Taylor, & Glover, 2006). In the following, the emphasis lies on prenatal depressive mood and depression.

Nowadays, depression, stress and anxiety are often studied together at the same time, as they are highly correlated with each other (Gerardin et al., 2011). It has to be noted that less research has been done on prenatal depression, than on prenatal anxiety (Van den Bergh et al., 2005) and prenatal stress in general.

5.1. Prevalence and measurement during pregnancy

In a newer review, prevalence rates for clinical depression have been reported to be substantial, namely 7.4% for the first, 12.8% and 12.0% for the third trimester of pregnancy (Bennett, Einarson, Taddio, Koren, & Einarson, 2004). Postpartum depression affects about 7.5% of the mothers (Rich-Edwards et al., 2008). Depressive mood (without fulfilling of all clinical criteria of a minor or major depression) seems to be much more common, with a prevalence of 25% pre- and 16% postpartum (Da Costa, Larouche, et al., 2000). The results of the review mentioned before point out a specific difficulty during pregnancy: Common diagnostic questionnaires (in this case the Beck Depression Inventory, BDI) reveal higher rates of depression than structured interviews or specifically designed questionnaires such as the Edinburgh Postnatal Depression Scale (EPDS) do. This is because depression related symptoms,

especially physical symptoms such as fatigue, are also accompaniments of pregnancy. Therefore, usual assessments of depression commonly overrate depression rates during pregnancy. A specific assessment tool is the already mentioned EPDS. Although it was originally developed for postpartum depression, it has also been validated for use during pregnancy (Bergink et al., 2011). In order to filter out real depressive symptoms, the use of a special questionnaire is indispensable.

It is also interesting to consider the reverse situation, looking at how pregnancy affects depression. It has been suggested that the demands of pregnancy, in combination with preexisting chronic stressors, might be responsible for higher depression rates during pregnancy, as well as altered hormone levels, especially higher concentration of sex steroids affecting brain areas involved in the regulation of mood (O'Keane & Marsh, 2007). These connections could also account for depressive mood or specific depressive symptoms and not for diagnosed major or minor depressions only.

The list of factors that may influence depressive mood and depression during pregnancy is long:

- Relationship adjustment (Whisman, Davila, & Goodman, 2011),
- higher use of emotional coping and higher trait as well as state anxiety and more hassles (Da Costa, Larouche, et al., 2000),
- previous depressions, being single, unplanned pregnancy, lack of social support, lower socioeconomic status, family violence, increased life stress, substance abuse (Bowen & Muhajarine, 2006),
- increased CRH levels in mid-pregnancy (Rich-Edwards et al., 2008) or lowered levels of CRH in early pregnancy (Susman et al., 1999), and
- prenatal medical procedures (Kowalcek et al., 2002).

5.2. Possible biological mediators

Elevated maternal cortisol in connection with perceived stress is related to altered physiological profiles of the newborns in ways that mimic maternal prepartum conditions: higher EEG activity in the right frontal lobe and lower tones of the vagus nerve (Field, Diego, &

Hernandez-Reif, 2006). Depressed pregnant women had higher cortisol, and lower dopamine and serotonin levels, an endocrine status that is later reflected by their newborns (Field et al., 2006). Cortisol has, therefore, been suggested as a possible mediator in the depression/preterm-birth-relationship (Field et al., 2006; Gawlik et al., 2010). Apart from cortisol, higher CRH levels have also been found in depressed pregnant women, at least among those in the second trimester (O'Keane et al., 2011).

Notably, the general changes in the hormones of the HPA during the course of pregnancy – namely the hypercortisolemia and the therefore following cortisol withdraw postpartum – are suspected to have a non neglectable influence on the occurrence of depression in this phase of life (Kammerer et al., 2006).

5.3. The impacts of prenatal depression on the course of pregnancy and delivery

Similar results as those depicted in the sections on stress and anxiety can be found for depression, i.e.; effects on pre-, peri- and postnatal complications (Field et al., 2006). Depressive mood or depression has been studied in connection with higher risk for preeclampsia (Kurki et al., 2000), intrauterine growth retardation or delayed/restricted fetal growth (Field et al., 2006; Gawlik et al., 2010; Hoffman & Hatch, 2000), low birth weight (Field et al., 2006) and shortened gestation length or preterm birth (Dayan et al., 2006; Gawlik et al., 2010; O'Keane et al., 2011). It should be noted that there are also controversial reports in which findings of an association among prenatal depression and low birth weight and preterm delivery are not found. (Berle et al., 2005).

Higher levels of depressive mood are, moreover, related to a negative value of the quality of attachment between mother and child during pregnancy (Hart & McMahon, 2006).

Considering fetal heart rate in response to a maternal stress task (Stroop test), the heart rate increases of the fetuses of depressed women (according to a DSM-IV diagnosis) were highest when compared to fetuses of women with anxiety disorders and fetuses of healthy women (Monk et al., 2004). The activity of the fetuses of women suffering prenatal depression show higher activity levels compared to fetuses of women not suffering prenatal depression (Field et al., 2006).

5.4. The impacts of prenatal depression on the well-being of mother and child after birth

It has to be noted that there have been recent studies in which no relationships between maternal depression and neonatal outcomes were found (Boedecs et al., 2011), whereas other studies do find connections among the depressed state of the mother and disturbed infant development in humans, such as higher anxiety scores, impulsivity and sleep problems at one year of age (Gerardin et al., 2011).

Gerardin et al. (2011) did, moreover, find gender specific effects of prenatal stress, depression and anxiety (in agreement with previous findings in animal studies), specifically reporting that boys of prenatally stressed mothers showed lower motor skills and, especially, more anxiety (compared to girls and infants of non-stressed mothers).

Together with late pregnancy anxiety (Zaers, Waschke, & Ehlert, 2008), prepartum depressive mood is the best predictor for postpartum depressive mood (Da Costa , Larouche, et al., 2000). Antenatal pregnancy and anxiety moreover predict parenting stress after birth three and six months after birth (Misri et al., 2010).

5.5. Treatment of depression (or other psychopathologies) during pregnancy

Whereas psychotherapeutic treatment of depression is always possible during pregnancy, the use of antidepressive psychopharmacology can be critical and the pharmacological treatment of severe major depression episodes may therefore be difficult (Bowen & Muhajarine, 2006; Gawlik et al., 2010; O'Keane & Marsh, 2007). Gawlik et al. (2010) state, that in this case, both treatment as well as non-treatment might be followed by negative side effects and that an early detection of the disorder is therefore crucial to prevent pre- and postnatal consequences. The side effects of psychopharmacological treatment during pregnancy include higher risks for spontaneous abortions, IUGR, malformations, lower birth weight, preterm birth, higher risk of cardiac defects in the offspring, hypertension of the neonates, or withdrawal symptoms of the neonates (Gawlik et al., 2010). The effects of the medications partially overlap the possible effects of depression and anxiety themselves. Women on antidepressive medication also showed a dampened cortisol awakening response (Shea et al., 2007).

The choice of treatment of psychiatric disorders during pregnancy does always have to be carefully balanced out. A supportive psychotherapeutical treatment is recommended (Bowen & Muhajarine, 2006). Other helping factors besides psychotherapy seem to be a healthy nutrition, adequate sleep duration and social support (Bowen & Muhajarine, 2006).

5.6. Summary on prenatal depression and depressive mood

Prenatal depression has not been studied as extensively as stress and anxiety have been, but the existing studies exhibit associations between depression, pregnancy and birth complications and with the postpartal well-being of the mothers. Hormones of the HPA-axis also seem to be involved in these connections, while psychosocial variables such as coping or relationship (adjustment) seem to influence depressive mood itself.

6. Implications for the experimental studies

The above-described connections show the importance of further research on psychosocial variables during pregnancy and their biological correlates and counterparts.

Stress, depression and anxiety do have their individual impacts, but all together they sum up to severe consequences for pregnancy, mother and child. It is necessary to assess all three of them simultaneously. HPA-hormones have been suggested to work as mediators or moderators of the connections of psychosocial variables and adversities, but the signaling pathways are still poorly understood. In order to better understand these mutual interactions, more thorough studies on the HPA-hormones and their relations to the relevant variables are necessary. Coping can directly add to the impact on the adversities, but as only certain coping styles are related to them, here too, further studies are very important. Their indirect influence or stress-buffering effects are still a matter of current research.

6.1. Aims of the experimental studies

This thesis was a part of a larger project named *Psychobiological stress-reactivity of second trimester pregnant women and their unborn children*, funded by the Swiss National Foundation SNF. The main aim of the entire project was to elucidate biological and psychological stress reactions of pregnant women and the fetus as well as the consequences of the stress reactivity and experience on the further course of pregnancy, on birth and the well-being of mother and child after birth.

In the following, the specific aims of the evaluation of the data for this thesis will be presented.

6.1.1. Study 1 - Coping styles in relation to perceived stress, birth anxiety and depressive mood during pregnancy

The possibly severe impacts of stress, anxiety and depressive mood on pregnancy have been elucidated above. Moreover, it has been shown that the way women cope during pregnancy might have a non-negligible impact on these psychosocial variables. While the prevalence of different coping styles as well as factors (such as socio-demographic variables) influ-

encing them have been widely examined, this study focused on the impact that different coping styles have on perceived acute stress, birth anxiety and depressive mood during pregnancy. Additionally, predominant coping styles have been highlighted and examined for their stability or change, respectively. The impact of change or stability has moreover been determined.

6.1.2. Study 2 - Second trimester amniotic fluid corticotropin-releasing hormone and urocortin in relation to perceived stress and anxiety during amniocentesis

The altered conditions considering HPA-hormones, their specific roles during pregnancy and their relation to stress and anxiety is a widely examined field of research, with respect to the maternal plasma levels of the said peptides. In contrast, little or nothing is known when it comes to amniotic fluid levels of the peptides in the context of stress and anxiety. While amniocentesis has been proven to be stressful for pregnant women, it is not only a standardized stressor but simultaneously presents a possibility to determine peptide levels in the amniotic fluid. These levels have been regarded in connection with perceived stress and anxiety in the second study of this thesis.

Part III. Experimental Studies

7. Coping styles in relation to perceived stress, birth anxiety and depressive mood during pregnancy

7.1 Introduction

Stress during pregnancy may have severe negative effects on the ongoing pregnancy and birth outcome (Austin & Leader, 2000; Copper et al., 1996; Roy-Matton et al., 2011; Schneider et al., 2011; Wisborg et al., 2008). Moreover, the well-being of the new mothers (Nierop, Bratsikas, Zimmermann, & Ehlert, 2006) and their children (de Weerth, van Hees, & Buitelaar, 2003) after birth have been shown to be related to prenatal maternal stress. Besides everyday stressors such as daily hassles, job and family stress and critical life events, pregnant women have to face additional challenges specifically related to pregnancy and future motherhood. The new field of prenatal diagnostics has opened opportunities for monitoring relevant parameters for the mothers-to-be, but research shows that these possibilities also may generate stress and anxiety. Amniocentesis is an invasive prenatal diagnostic procedure bearing an average complication rate of 0.74% (Bettelheim et al., 2002). Like with other prenatal screenings, it is a stressful, anxiety-promoting event for pregnant women (Brajenović-Milić et al., 2010; Kowalcek et al., 2002; Marteau et al., 1992; Pauli, Blaser, & Hermmann, 2008). The perceived distress has been found to be both due to the procedure itself and the possibility of negative results of the screening (Marteau et al., 1992).

In addition to stress, depression and anxiety are also related to gestational complications i.e. risk for preeclampsia, (Kurki et al., 2000). Concerning psychological well-being, both antenatal anxiety and antenatal depression precede the same symptoms in the post partum period (Heron et al., 2004). In nulliparous women, fear of childbirth was shown to be related to the lack of adequate psychosocial resources and adverse sociodemographic factors such as a low educational levels, little social contacts and unskilled jobs or unemployment on one hand and psychological factors such as high general anxiety and depressive symptoms on the other hand (Laursen et al., 2008). Fear of childbirth has also been stated as predictive for a negative psychological status of the mothers after birth (Sieber et al., 2006). Therefore, pregnancy related stress, anxiety and mood may adversely impact the course of pregnancy (Dunkel-Schetter, 2011).

Previous research of our work group has chosen a resource based approach and has clearly shown that psychosocial resources serve as stress-buffers during pregnancy in a way that

higher resources were associated with lower psychophysiological stress responses and higher mood levels (Nierop et al., 2008). Additionally, it has been shown that emotional well-being changes during the course of pregnancy, especially in the final trimester, resulted in a higher degree of confidence in coping with labor and giving birth (Sieber et al., 2006). As a continuation of this, the present study focuses on coping strategies (as suggested by Nierop et al., 2008) and the stability or change in coping styles across pregnancy in connection with well-being of the pregnant women.

7.1.1. Coping during pregnancy

Coping during pregnancy has been examined in samples of high risk (i.e. Yali & Lobel, 1999), mixed medical risk (Hamilton & Lobel, 2008) as well as in healthy pregnant women (Borcherding, 2009; Huizink et al., 2002a). Especially sociodemographic factors such as age, income and education have shown to be strongly related to coping styles during pregnancy (Borcherding, 2009; Yali & Lobel, 1999). The use of positive appraisal when faced with adversities seems to be related to less distress during pregnancy (Yali & Lobel, 1999, 2002). The use of emotion-oriented coping, is associated with higher anxiety during pregnancy and lower emotional well-being in the pre- and postnatal period (Da Costa, Larouche et al., 2000). A lack of coping strategies for stressful situations is related to adverse pregnancy outcomes, i.e. low birth weight (Borders et al., 2007).

The importance of healthy and sufficient coping skills has led to the endeavor of identifying factors influencing the way expectant mothers manage stressful events.

In current literature, different coping inventories are used to assess coping styles of pregnant women (Dunkel-Schetter, 2011). The following coping measures are used or revised in studies with pregnant women (Literature using the specific assessment in brackets): Coping Inventory for Stressful Situations (CISS) (e.g. Borcherding, 2009), Prenatal Coping Inventory (PCI) (e.g. Yali & Lobel, 1999) or certain items of the PCI (e.g. Borcherding, 2009), the Revised Prenatal Coping Inventory (NuPCI) (e.g. Hamilton & Lobel, 2008), the Utrecht Coping List (UCL-19) (e.g. Huizink et al., 2002a), the Coping Responses Inventory (CRI) and the Ways of Coping Questionnaire (WCQ) (both e.g. Ayers, 2001). The wide variety of the used assessment instruments reveals a difficulty in the comparability of previous research.

7.1.2. The present study

The main interest of this study was to investigate the possible relations of applied coping strategies with perceived burden, anxiety and depressive symptoms at different time points during pregnancy. Stress, anxiety and depression may have strong effects on the course of pregnancy, birth outcomes and well-being of mother and child. Therefore, the investigation of the way pregnant women cope with such adversities has become an intense field of interest. If it is possible to identify a connection between certain coping strategies and adversities such as perceived stress, anxiety and depression during pregnancy, such connections might suggests paths to prevention programs especially designed for pregnancy.

Therefore, the present study has three main aims:

First, to investigate the influences of different coping styles on perceived stress in a pregnancy related stress situation in healthy pregnancies.

Second, to examine the influence of the predominant coping styles on birth anxiety and depressive mood in the second as well as in the third trimester. We want to elucidate possible differences in the impact of coping styles on mood and pregnancy related anxiety in the different trimesters.

Third, to explore the stability or change of coping strategies during the course of pregnancy. Additionally, the possible impact of stability or change in the predominant coping styles is investigated.

7.2. Methods

7.2.1. Participants

Pregnant women were recruited from patients being advised and/or treated in the departments of gynecology and obstetrics of the University Hospital of Zurich, the Hospital of Wetzikon and the Cantonal Hospital of Lucerne between April 2009 and December 2010. As amniocentesis is an invasive procedure bearing a risk for complications (Bettelheim et al., 2002), only women assigned for the procedure for medical reasons or reasons of age were eligible for the study.

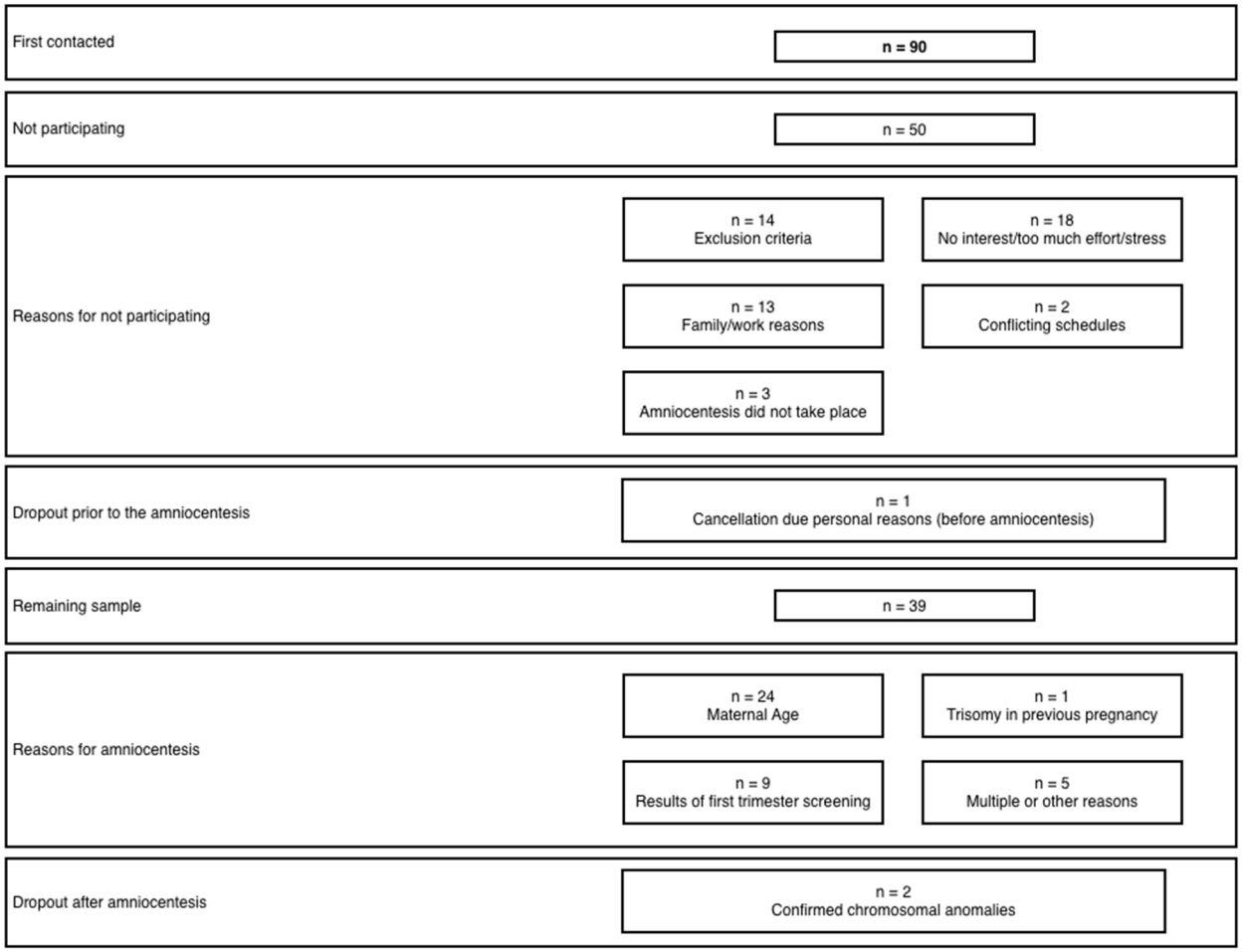
7.2.1.1. Exclusion criteria

Exclusion criteria were the following: artificial insemination, known medical complications (concerning the mother-to-be or the fetus), suspected or known fetal growth restriction, ultrasound confirmed fetal structural anomalies, psychiatric disorders of the pregnant women, alcohol intake (more than one standard drink per week), current medication (glucocorticoids, psychotropic drugs, diuretics, antihypertensive, vasodilators), food and/or protein restrictions and insufficient language knowledge to fill in the questionnaires.

7.2.1.2. Sample

In total, 90 women were contacted and invited to be participants in the study. 51 women did not participate. The total recruiting procedures with exclusions and inclusions is presented in Figure 7.1. This resulted in 39 healthy pregnant women with a singleton intrauterine pregnancy being included into the study.

Figure 7.1. Sampling process



Note. The recruiting process of the participating women from the first contact (phone screening) to the accomplished amniocentesis.

7.2.2. Ethics

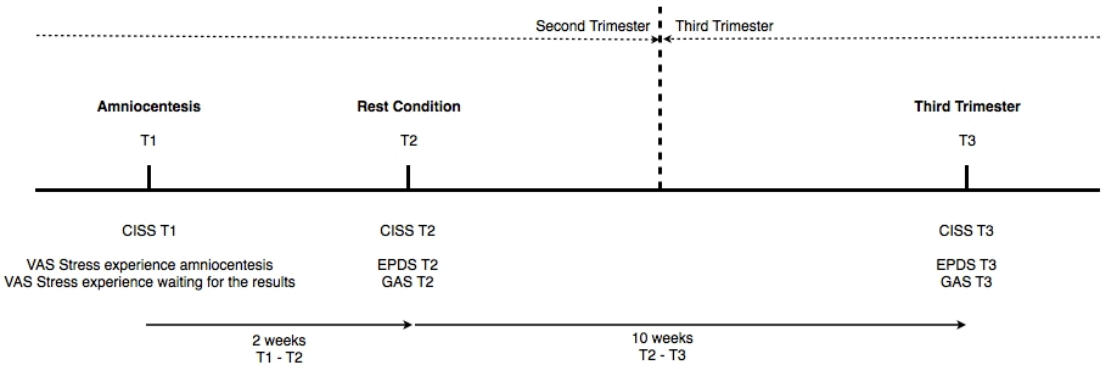
The study protocol was approved by the ethics committees of the Canton of Zurich and of the Canton of Lucerne. Every woman signed written informed consent before entering the study procedure.

7.2.3.Procedure

7.2.3.1. Assessment time points.

Data were assessed at three different time points during pregnancy. The first assessment time point was the day of the amniocentesis in the second trimester of pregnancy. The second assessment time point was a stress-free rest condition, also in the second trimester. The rest condition took place two weeks after the stress condition, because the diagnostic results were obtained at this time and it was necessary to assure that worrying about the results did not influence the condition. The third assessment time point was during the third trimester of pregnancy approximately 10 weeks after the rest condition. Questionnaires were filled out by the women at home. The exact design is shown in Figure 7.2.

Figure 7.2. Timeline



Note. CISS = Coping Inventory for Stressful Situations. VAS = Visual analogue scale. EPDS = Edinburgh Post-natal Depression Scale. GAS = Geburtsangst-Skala [Birth Anxiety Scale].

7.2.3.2. Psychological measures

7.2.3.2.1. Stress experience – Amniocentesis

The intensity of the stress experience during amniocentesis (SEA) itself as well as stress caused by the waiting for the results was assessed using visual analogue scales. Each response was given by placing a cross on a 100mm line; with a continuum from 0 (*not at all*) to 100 (*highest possible*). The questions concerning amniocentesis included actual subjectively perceived anxiety, stress, arousal, personal challenge and the urge to leave the situation, namely (1) current nervousness, (2) current stress-level, (3) desire to leave the situation immediately, (4) challenge of the situation, (5) current general anxiety and (6) current anxiety due to the procedure.

The questions were asked repeatedly at 40, 15 and 1 minutes before the amniocentesis and mean scores were computed over time.

7.2.3.2.2. Stress experience - Waiting for the results.

In order to assess perceived stress due to the waiting for the results, pregnant women were asked for their (1) anxiety about a possible negative result and (2) the discomfort due to the waiting for the results.

The questions were also asked at 40, 15 and 1 minutes before and additionally 20 minutes after amniocentesis.

7.2.3.3. Coping

Coping was assessed by using a German short version of the Coping In Stressful Situations Scale (Endler & Parker, 1990, Kälin, 1995). This version of the CISS consists of 24 items describing typical behaviors during stressful situations. The CISS is a multidimensional assessment tool, meaning that it is possible to score on every subscale. The answers are given on a five-point Likert-type scale ranging from (1) *very unlikely* to (5) *very likely* and describe the possibility that the given behavior would be performed. The CISS has 3 main subscales, namely *Task, Emotion* and *Avoidance*. *Task coping* (TC) covers strategies including focusing on priorities, thinking how one solved similar problems in the past and planning. *Emotional coping* (EC) is constituted of strategies such as accusations towards oneself, fear of not being able to manage the situation or of being taken aback. *Avoidance coping* (AC) is characterized by social and other distractions, such as shopping, eating or watching a movie.

Mean scores - instead of sum scores - were computed for all subscales, as suggested by Kälin (1995).

7.2.3.4. Birth anxiety

To assess birth anxiety, we used a German birth anxiety scale, the "Geburtsangst-Skala" (GAS), by Lukesch (1983). The GAS is a 77-item questionnaire asking about fear of different situations possibly occurring during pregnancy and birth. The women are instructed to imagine the depicted situations vividly and to give the answer according to their perceived anxiety on a four-point Likert-type scale ranging from (0) *I am not anxious at all* to (3) *I am very anx-*

ious. For interpretation, a total sum score was computed.

7.2.3.5. Depressive mood

Depressive mood was assessed using a German Version of the Edinburgh Postnatal Depression Scale (Bergant, Nguyen, Heim, Ulmer, & Dapunt, 1998; Cox, Holden, & Sagovsky, 1987). The questionnaire has also been evaluated to be used during pregnancy (and not only in the post partum period) to assess depression related symptoms (Bergink et al., 2011). Regular diagnostic tools for depression often overrate depression during pregnancy, given that certain symptoms usually assigned to depression can also occur during healthy pregnancy, such as fatigue. The EPDS consists of ten items addressing the psychological well-being of the subject during the past week. Answers are given on a four-point Likert-type scale with point descriptions varying from question to question. A total score from 0-30 is possible, while 0-9 indicates that the probability of depression is low, 10-12 indicates a moderate probability and scores of 13 and over indicate a high probability of depression when assessed postpartum. Bergink et al. (2011) suggest a cut-off level of 10 if assessed during the second trimester of pregnancy.

7.2.4. Statistical Analyses

Statistical Analyses were performed by using IBM SPSS Statistics 19 for Mac OS X.

Possible confounders: Previous research has shown, that several sociodemographic factors, namely age, income and education level, are commonly related to coping styles (Borcherding, 2009; Yali & Lobel, 1999). Therefore, prior to the analyses, these possible influences were investigated using correlation analyses. Moreover, one might assume that stress experience due to amniocentesis might vary corresponding to the specific reasons the prenatal diagnostics were performed for (for the different reasons, see Figure 7.1). Therefore, the possible confounding influence was analyzed using analyses of variance (ANOVA).

Coping in relation to perceived stress, birth anxiety and depressive mood To test the influence of different coping styles on perceived stress in a pregnancy related stress situation, as well as on anxiety and depressive mood, bivariate correlations (Pearson's correlation coefficients) were computed. To check for the predictive values of the independent values (namely

the different coping strategies) on the dependent variables (namely the SEA and the SEW), two separate linear analyses were performed.

Predominant coping styles The women were assigned to a predominant coping style based on their scores on the CISS subscales. If the scores in two different subscales were equal, both coping styles were regarded as predominant. Due to the greater stability of non-parametric tests when examining groups with large differences in group size, the Kruskal-Wallis-Test was chosen for testing the effect of predominant coping styles at different time points on birth anxiety and depressive mood.

Stability of coping For testing the possible influence of a stable versus an unstable predominant coping style during the course of pregnancy, Kruskal-Wallis-Tests were performed for the reasons discussed above.

7.3. Results

7.3.1. Dropout analyses

Of the 39 women initially participating, 23 answered the CISS questionnaire in the third trimester (dropout rate of approximately 41%). To check for a probable selective dropout, women participating in both trimesters and women participating in the second trimester only were compared with respect to all possibly relevant variables using t-Tests. There were no significant differences; a selective dropout could therefore be ruled out.

7.3.2. Possible confounders

Age, income and education level were not associated with the assessed coping styles in our sample. This might be explained by the high homogeneity of the Swiss sample, mainly consisting of women with small differences in socioeconomic factors.

Concerning the reasons the prenatal screening was performed for in relation to the perceived stress, the performed tests showed no significant differences in our sample.

As no significant effects could be shown, the variables were not controlled for.

7.3.2. Descriptive analyses

7.3.2.1. Sample

The pregnant women were 27 to 45 years old with a mean age of 37.65 years (*SD* = 4.11 years). The amniocentesis was, on average, performed on the 110th day of pregnancy (range 100-116 days, *SD* = 3.76 days).

7.3.2.3. Descriptives of all coping styles, birth anxiety and depression

Descriptive data for the CISS, the GAS and the EPDS results are summarized in Table 7.1.

Table 7.1. Descriptive data for the coping subscales, birth anxiety levels and depressive symptom scores at the different time points

		T1		T2		T3	
Questionnaire		*M* ± *SD*	Range	*M* ± *SD*	Range	*M* ± *SD*	Range
CISS	TC	3.89 ± .59	2.75-5.00	3.89 ± .68	2.38-5.00	3.70 ± .78	1.88-5.00
	EC	2.76 ± .69	1.00-4.25	2.63 ± .65	1.25-3.71	2.75 ± .69	1.25-4.00
	AC	3.21 ± .82	1.25-4.63	3.02 ± .83	1.13-4.25	2.82 ± .87	1.00-4.75
GAS		-	-	70.69 ± 43.73	8.00-179.00	68.13 ± 47.93	5.00-202.00
EPDS		-	-	4.87 ± 4.17	0-16	6.75 ± 6.22	0-22

Note. The means (*M*), standard deviations (*SD*) and ranges of the coping styles (TC = task coping, EC = emotion coping, AC = avoidance coping), birth anxiety (GAS scores) and depressive mood (EPDS scores) at all three assessment time points (T1 = amniocentesis, T2 = rest condition, T3 = third trimester).

7.3.2.3. Predominant coping styles at the three time points

Most women were predominantly using task coping strategies throughout the course of pregnancy, regardless of the stress or rest condition (T1 76.3%, T2 78.4% and T3 60.9%, respectively). Detailed descriptives of predominant coping styles can be seen in Table 7.2.

Table **7.**2. Predominant coping styles at all three time points

	Second Trimester				Third Trimester	
	T1 (amniocentesis)		T2 (rest condition)		T3	
Coping styles	Frequency	Valid percent (%)	Frequency	Valid percent (%)	Frequency	Valid percent (%)
Avoidance	3	7.9	3	8.1	2	8.7
Emotion	3	7.9	2	5.4	2	8.7
Task	29	76.3	29	78.4	14	60.9
Avoidance/Task	2	5.3	2	5.4	2	8.7
Emotion/Task	1	2.6	1	2.7	2	8.7
Avoidance/Emotion	-	-	-	-	1	4.3
In Total	38		37		23	
Missing (System)	1		2		16	
Total	39		39		39	

Note. Women were assigned to their predominant coping style(s) according to the coping style or the coping styles they most frequently used according to the CISS subscales (avoidance, emotion, task). If the scores of two subscales appeared to be equal, both coping styles were assumed to be predominant (avoidance/task, emotion/task, avoidance/emotion).

7.3.3. Influence of coping style on stress experience

At the onset, the results of the correlation analyses of the coping variables and the stress experience scores are depicted. TC had no significant correlations with any of the stress experience scores. A protective effect, as might be assumed, could not be shown in our sample according to the methods by which the AC was operationalized. As no significant associations were revealed, it was not included in the regression analyses. All correlations are depicted in Table 7.3.

Table 7.3. Correlations of perceived stress in connection with amniocentesis and waiting for the results

	(1)	(2)	(3)	(4)	(5)
EC (1)	-	.112	.016	.378*	.160
AC (2)		-	.370*	.290	.421**
TC (3)			-	-.034	-.015
SEA (4)				-	.621**
SEW (5)					-

Note. Correlations of the coping styles (TC = task coping, EC = emotion coping, AC = avoidance coping) with stress experience related to the amniocentesis (SEA) and the waiting for the results (SEW).
*$p < .05$, **$p < .01$, ***$p < .001$

7.3.3.1. Impact of different coping strategies on perceived stress due to amniocentesis and waiting for the results

In order to examine the relation between coping style and perceived stress during an acute stressor, two separate linear regression analyses were performed for perceived stress during the amniocentesis and while waiting for the results. EC and AC were entered at the same step.

For the stress caused by amniocentesis, the model with EC and AC as predictors was significant with $R^2 = .205$ and $F = 4.521$, $p = .018$. *Betas*, *Standard Errors of Betas* and standardized *Beta* can be seen in Table 7.4. A higher use of EC went along with higher reported stress during the amniocentesis procedure ($r = .350$, $p < .03$). In total, the predictors explained 20.5% of the variance of perceived stress concerning amniocentesis.

Table 7.4. Summary of the regression analyses for the prediction of stress experience caused by the amniocentesis and the waiting for the results by emotional and avoidance coping

	Amniocentesis			Waiting for the results		
Stress experience	*B*	*SE B*	*β*	*B*	*SE B*	*B*
EC	9.586	4.125	.350*	3.849	5.132	.114
AC	5.777	3.488	.251	11.484	4.311	.408*
R^2		.205			.190	
F		4.521*			4.104*	
p		.019			.025	

Note. Amniocentesis: $R^2 = .205$, $F = 4.251$, $p = .019$. Waiting for the results: $R^2 = .190$, $F = 4.104$, $p = .025$.
*$p < .05$, **$p < .01$, ***$p < .001$

The regression model for EC and AC as predictors for the subjective stress caused by the waiting for the results was significant ($R^2 = .190$, $F = 4.104$, $p < .03$, for *B*s, *SE B*s and *β* see Table 7.4). In this case, the use of more AC strategies predicted higher experienced stress due to the waiting for the results. The predictors explained 19.0% of the variance of the cumulative stress score concerning waiting for the results.

7.3.4. Associations of different coping strategies with birth anxiety and depressive mood in the second and third trimester

AC and TC showed no significant correlations with the variables at the focus of our interest: neither in the second nor in the third trimester of pregnancy.

EC at T3 showed a high positive relation with birth anxiety ($r = .76$, $p < .001$) and depressive mood ($r = .71$, $p < .001$) in the third trimester, while there were no significant correlations in the second trimester.

7.3.5. Predominant coping styles and their relation to birth anxiety and depressive mood in second and third trimester

As the groups for predominant AC, predominant EC and mixed predominant coping styles were very small (1-3 participants), we decided to compare predominant TC vs. any other pre-

dominant coping style using t-Tests.

For the second trimester of pregnancy, no significant differences in birth anxiety or depressive mood for women predominantly using TC compared to other predominant coping style could be shown. For the third trimester, however, it could indeed be shown that women with a predominantly task-oriented coping style had significantly lower depressive mood levels than women using an alternative predominant coping styles ($p < .05$). These relations did not apply for birth anxiety in the third trimester ($p = .08$).

7.3.6. Stability vs. change of predominant coping styles during pregnancy in relation to depressive mood and birth anxiety

Details on stability, respectively change, of the predominant coping style can be seen in Table 7.5. It shows that 83.3% of the women did not change their coping style from the stress condition (T1) to the rest condition (T2) in the same trimester.

Table 7.5. Stability of the predominant coping styles

	T1 – T2	T2 – T3	T1 – T3	T1 – T2 – T3
	Valid percent (n)	Valid percent	Valid percent	Valid percent
Stable	83.3 (30)	50.0 (12)	60.9 (14)	43.5 (10)
Change	16.7 (6)	50.0 (12)	39.1 (9)	56.5 (13)
In total	36	24	23	23
Missing	3	15	16	16
Total	39	39	39	39

Note. Stability analysis across all three assessment time points (T1 = amniocentesis, T2 = rest condition, T3 = third trimester).

43.5% of the women remaining in the study for both trimesters showed a stable predominant coping style throughout all three assessment time points. These women showed significantly lower depressive mood levels at T2, but not at T3 – compared to women who changed their predominant coping style. In all the other conditions, change or stability in the predominant coping style did not have a significant influence on depressive mood and birth anxiety in the second and third trimester.

7.5. Discussion

The main aims of this study were, during pregnancy: 1. to investigate the relationship between several different coping styles and perceived acute stress, 2. to explore possible connections between predominant coping styles and depressive mood/birth anxiety and 3. to evaluate the stability of coping styles across the entire period of pregnancy.

Most of the women in our study were assigned to a predominant TC throughout the entire pregnancy. The means for TC strategies were highest at both assessment time points in the second and in the third trimester. This result is in line with (Da Costa, 1997), who also used the CISS to assess coping during pregnancy and reported the highest means for TC compared to all other subscales across pregnancy . Hamilton & Lobel (2008) used a different coping assessment specifically on coping strategies during pregnancy but found that planning-preparation was more consistently used throughout the course of pregnancy. Borcherding (2009) reported prayer and task coping as the coping strategies used most often during pregnancy.

We were able to show that a more frequent use of EC during the amniocentesis was associated with a higher reported perceived stress in the situation itself, an observation that goes in line with previous research generally associating EC with higher levels of distress (Folkman & Moskowitz, 2004). A more frequent use of AC instead, was related to higher perceived stress due the waiting for the results. This finding is interesting in association with previous studies. Yali & Lobel (1999) indeed also found a connection between AC and greater pregnancy specific stress; this was confirmed by a newer study reporting associations of AC with high state anxiety and pregnancy specific stress, such as worrying about the baby's health (Hamilton & Lobel, 2008).

The coping style with the most negative impact on psychological well-being seemed to be emotional coping. A more frequent use of EC strategies (regardless whether it was a predominant coping style or not) was related to higher perceived acute stress as well as higher birth anxiety and depressive mood levels during the third trimester. These results are again in line with previous research using CISS for coping assessments where it has been found that women with depression during pregnancy used more emotional coping compared to a non-depressed control group (Da Costa, 1997; Da Costa, Larouche, et al., 2000). They did not find

any effects of task and avoidance oriented coping strategies on depressive symptoms as well.

While the major part of the participating women reported the same coping style at the stress condition and the stress-free rest condition (83.3%), 43.5% of the women completing the study showed stable predominant coping styles across the entire study period. Interestingly, women who changed their predominant coping style at least once throughout our assessment time period showed a higher depressive mood level in the second trimester compared to women who stayed stable concerning their predominant coping style at all measurement time points. Whether the change of the predominant coping style is a precursor or a cause of the higher depressive mood cannot be determined from our data and would have to be the subject of investigation in future studies.

The fact that we did find relations of different coping strategies with depressive mood and birth anxiety during the third, but not in the second trimester will have to be the focus of future research. It might suggest that different pathways of moderating effects work throughout the course of pregnancy.

7.5.1. Limitations of the study

Our sample was very homogenous with regard to age, socioeconomic status and education level, which made it unnecessary to control for those variables. Because of this, perhaps, depressive mood and birth anxiety level were relatively low in the entire sample, along with the most frequent use of task coping strategies. For further studies, a more heterogeneous group concerning the variables in focus would be desirable. Additionally, for purposes of reproduction of these results and possibilities of generalization, a bigger sample size should be chosen for further studies, as a larger sample size of a less homogenous sample might make for better generalization of the results.

In our sample, 23 or 59% of the pregnant women provided data at all measurement time points. The dropout-rate of 41% is relatively high, but still in the range of previous long-term studies on coping during pregnancy (Da Costa, Larouche, et al., 2000: 17%; Hamilton & Lobel, 2008: approximately 47%; Huizink et al., 2002: 25%). Although the drop-out participants did not differ in the variables in focus from the rest of the sample, a steadier sample is desira-

ble for the long term analyses considering stability of coping. Therefore, in-house appointments during the third trimester might help to lower the rate of missing data during this period of pregnancy.

7.5.2. Implications for further research

Our study has shown that coping may have an important impact on the psychological well-being of pregnant women. As stress, anxiety and depression are related to pregnancy and birth complications as well as birth outcome, a more thorough observation of coping strategies is indicated. A better understanding of coping and factors influencing coping during pregnancy might help to develop prevention campaigns and programs which in turn could lower anxiety and depressive mood rates among pregnant women.

For further research, several topics should be addressed very carefully:

Operationalization of coping during pregnancy While some assessments such as the UCL-19, CISS, CRI and WCQ are general coping questionnaires; the PCI and it's revised NuPCI are questionnaires for pregnancy-specific coping. The authors of the latter instruments insist that general coping scales are not sufficient for the assessment of coping under the special circumstances of pregnancy (Yali & Lobel, 1999). The pregnancy-specific questionnaires focus on preparation for the baby, talking about the pregnancy or praying for an uncomplicated birth or a healthy pregnancy (Borcherding , 2009). In our study, coping was first assessed in an acute stress condition, making the use of a general instrument indispensable. Nevertheless, in order to capture both pregnancy-related and general coping styles it might be helpful for future studies to apply both specific and general instruments as, for instance, was the case in the study of Borcherding (2009).

Effects of amniocentesis on anxiety and depression Whereas the effects of the procedures and the risk for a negative result have been well studied with similar results, the long term effects of prenatal screenings are still the subject of active debate. Marteau et al. (1989) have shown that women who underwent prenatal screening have lower third trimester anxiety levels and a more positive attitudes towards pregnancy at this time than women without a screening. In a later publication, Marteau et al. (1992) reported more worries about and a less positive attitude towards the baby after birth compared to women without prenatal diagnostics,

although the authors explain these findings with pre-existing group differences.

The anxiety and depressive mood levels in our sample are relatively low, even if the EPDS cut-off level for postpartum women is adjusted for prepartum women (Bergink et al., 2011). As there is no control group without prenatal diagnostics, a long-term impact of the procedure may not be entirely excluded.

7.5.3. Summary and perspective

Our findings show that the use of different coping strategies has an impact on perceived stress, depressive mood and birth anxiety during pregnancy. The results suggest that the predominant use of task coping during the third trimester might be protective against depressive moods. A greater use of emotional coping strategies, on the contrary, was related with higher stress experience during amniocentesis and birth anxiety and depressive mood during the third trimester, suggesting that excessive use of emotional coping strategies might be dysfunctional during the course of pregnancy. Further research is necessary to confirm the results. As stress, depressive mood and anxiety are related to pregnancy complications and outcomes, extended knowledge about the influence of coping strategies might help to develop prevention campaigns and programs.

8. Second trimester amniotic fluid corticotropin-releasing hormone and urocortin in relation to perceived stress and anxiety during amniocentesis

8.1. Introduction

Stress has been shown to be related to pregnancy complications, adverse birth outcome (Austin & Leader, 2000; Copper et al., 1996; Roy-Matton et al., 2011; Schneider et al., 2011; Wisborg et al., 2008) and to the negative well-being of the mother and the newborn after birth (de Weerth et al., 2003; Nierop, Bratsikas, Klinkenberg, et al.,2006). Since some in the stress response involved peptides such as CRH and UCN also play a major role during human pregnancy, it is necessary to achieve a better understanding of possible pathways connecting psychological stress and physiological adversities during human pregnancy.

The corticotropin-releasing hormone (CRH), a 41-amino acid peptide, is widely known for its orchestrating role in the endocrine stress response. CRH is released by the hypothalamus and mainly acts through the CRH-1-receptor (Joëls & Baram, 2009) as a stimulator for the hypothalamus-pituitary-adrenal axis (HPA) to release adreno-corticotropin-releasing hormone (ACTH) from the pituitary, leading to the secretion of glucocortiocids from the adrenal cortex (Bale & Vale, 2004; Tsigos & Chrousos, 2002). A controlling negative feedback mechanism (including ACTH and cortisol) inhibits the further release of CRH and causes the stress response to shut down (Charmandari et al., 2005).

Urocortin (UCN) is a peptide of the CRH-family consisting of 40 amino acids (Latchman, 2002). It has a 45% homology with CRH (Vaughan et al., 1995). It was first characterized 16 years ago and binds to both CRH-1- and CRH-2-receptors, to the latter one with a higher potency than CRH itself (Vaughan et al., 1995). Apart from binding to the same receptor, both CRH and UCN are highly affiliated to the CRH-binding protein (Florio et al., 2004). UCN is also known for its ACTH-stimulating capability, but as it binds to both CRH-1 and -2-receptors; it has been suggested to play an intermingled role in the stress reaction (Bale & Vale, 2004), as CRH-1-receptor mediated actions generally have activating and stimulating effects within seconds or minutes after the onset of the stressor, while the CRH-2-receptor mediated long-term actions are involved in the shutting down of the stress response as well as lasting adapting and consolidating effects (Joëls & Baram, 2009). The two other members of

the CRH-family, UCN II and UCN III, specifically bind to the CRH-2-receptor and are considered to play an important role in dampening the stress sensitivity, as it might also be the case with UCN (Bale & Vale, 2004). Recent animal studies suggest a prominent role for UCN in the stress recovery process, showing that triple knockout (UCN, human stresscopin and stresscopin-related peptide) mice exhibited more anxiety-related behavior 24 hours after, but not immediately after being exposed to a stressor (Neufeld-Cohen et al., 2010).

CRH has at least two major functions during human pregnancy and labor. On the one hand it keeps the myometrium from contracting during gestation (Torricelli, 2006), on the other hand it functions as a placental clock by triggering birth onset (Hillhouse & Grammatopoulos, 2002; McLean et al., 1995). UCN structurally and biologically highly resembles CRH and is expressed in various reproductive organs and tissues as well, such as the myometrium, endometrium, the decidua and the placenta; it plays an important role during human pregnancy (Florio et al., 2004). Both hormones stimulate placental ACTH secretion, regulate placental blood flow and vasodilatation, are involved in uterine contractility and relaxation and play a role in fetal processes and in the development of fetal adrenal function and the maturation of organs (Florio et al., 2004; Hillhouse & Grammatopoulos, 2002; Petraglia, 1999; Petraglia et al., 1987).

CRH rises throughout the course of pregnancy, starting from the second trimester and culminating in a peak at term whereas the CRH-binding-protein decreases (Campbell et al., 1987; Florio, Zatelli, Reis, & Uberti, 2007). Therefore, CRH and its homologue UCN have been studied for their role in preterm birth. Elevated plasma levels of both peptides have been shown to be related to preterm birth (Dunkel-Schetter, 1998; Florio et al., 2007; Hillhouse & Grammatopoulos, 2002; Torricelli, Voltolini, Galleri, et al., 2009; Vogel, Thorsen, Curry, Sandager, & Uldbjerg, 2005). Moreover, CRH and the experience of subjective stress seem to be predictive for preterm birth (Dunkel-Schetter, 1998) although the exact pathways of this process still remain unclear. In consequence, the relationship of CRH and subjective stress during pregnancy has been studied, with results that, at the present time, remain the subjects of discussion (Chen et al., 2010).

Although CRH seems to be associated with subjective stress experiences during pregnancy and may be predictive of preterm birth (Dunkel-Schetter, 1998), research findings to date are

controversial. Chen et al. (2010) report a negative association between CRH in maternal blood and psychosocial stress measures. Hobel, Dunkel-Schetter, Roesch, Castro, and Arora (1999) report the same negative correlations for stress and CRH in a group of pregnant women who delivered at term, while positive associations were found for a group of women who delivered preterm. Mancuso et al. (2004) did not find a significant correlation of perceived stress and plasma CRH neither in the second nor in the third trimester of pregnancy. Apart from its actions during pregnancy and birth, CRH seems to be involved in fetal programming (Weinstock, 2008; Welberg & Seckl, 2001) with possible consequences for child development such as altered neurotransmitter activity and temperamental difficulties in infants (Weinstock, 2005).

Only a few studies have assessed CRH and UCN in amniotic fluid. Amniotic fluid CRH levels are up to twenty-fold lower than the levels of the peptide in maternal plasma (Stalla et al., 1989). After the well studied connection between maternal plasma CRH and preterm birth, only few studies have also investigated the role of amniotic fluid CRH and UCN as promoters of preterm delivery (CRH and/or UCN; Florio et al., 2008; Iavazzo, Tassis, et al., 2009; Menon et al., 2008; Torricelli, Voltolini, Galleri, et al., 2009). In a retrospective study, Torricelli, Voltolini, Galleri et al. (2009) showed that amniotic fluid CRH levels were not related to preterm delivery at this point in time. This finding with regard to amniotic fluid CRH is surprising, as plasma CRH has been thoroughly investigated in this context and it has been shown that elevated plasma CRH levels are related to preterm labor (Fadalti et al., 2000; McLean & Smith, 2001; Schulkin , 1999) and birth (Hobel et al., 1999; Kalantaridou et al., 2010; Korebrits et al., 1998; Sandman et al., 2006). Moreover, already second trimester plasma CRH levels enable a distinction of pre-, post- and at term deliveries (McLean et al., 1995).

Regarding UCN, it has been reported that low amniotic fluid UCN levels during the second trimester serve as a predictor for preterm delivery (Torricelli, Voltolini, Galleri, et al., 2009). Iavazzo, Tassis et al. (2009), however, could not find a predictive value of UCN levels (retrieved during the second trimester at genetic amniocentesis) neither for preterm labor nor preterm birth. The authors themselves pointed to the controversial results, calling for further studies with bigger sample sizes and better exclusion criteria (Iavazzo & Malamitsi-Puchner, 2010). On the other hand, they suspected differences in the study design, methods of biochemical analyses and sample storage as possible reasons for the diverging findings.

Moreover, increased amniotic fluid CRH levels are brought into a connection with intraamniotic infection and inflammation at term (Florio et al., 2008). Amniotic fluid levels of UCN have been shown to be lower in women carrying a fetus with Down syndrome (trisomy 21) than in those experiencing healthy pregnancies, suggesting that these altered levels lead to a diminished neuroprotection (Torricelli, Voltolini, Biliotti, et al., 2009).

In connection with maternal psychological factors, CRH and UCN are known to play a role in the development of anxiety and anxiety related behavior mainly through the CRH-1-receptor (Bale & Vale, 2004; Dautzenberg & Hauger, 2002). Similar to the effects of stress, based on current research, it has to be assumed that the fetal (HPA) system is influenced by maternal anxiety as well: It has not only been shown that higher maternal anxiety is related to a higher risk of pregnancy complications such as preeclampsia (Kurki et al., 2000), but also to fetal intrauterine growth retardation (Gawlik et al., 2010) or preterm birth (Dole et al., 2003; Gawlik et al., 2010; Orr et al., 2007) and lower APGAR scores of the babies after birth (Berle et al., 2005). A recent study was able to show a connection between maternal pregnancy specific anxiety and higher stress reactivity of the babies after birth (Tollenaar et al., 2011), while general maternal anxiety seems to be related to an altered HPA-activity in pre-adolescent children (O'Connor et al., 2005) and attenuated diurnal cortisol profiles in adolescents (Van den Bergh et al., 2008).

Florio et al. (2004), described UCN as a substitute or mimic for CRH. Due to the high resemblance (Bale & Vale, 2004) in structure and pharmacological effectiveness, it has moreover been stated that previously CRH-attributed effects during pregnancy might in fact be UCN-mediated (Clifton et al., 2000). At any rate, a ‘characteristic interplay’ of CRH and UCN is suspected (Iavazzo, Baka, et al., 2009). The major roles in stress, pregnancy and anxiety, the high structural and pharmacological resemblance and at the same time the highly specific differences in functioning mechanisms, especially during the human stress response, underline the importance of measuring both CRH and UCN at the same time for a more specific evaluation.

8.1.1. Aims of the study

The main aims of the study were to investigate:

First, the relationships between amniotic fluid levels of CRH and UCN and subjectively perceived stress during amniocentesis in healthy pregnancy and

Second, the association between maternal trait anxiety at a stress-free rest condition and amniotic fluid CRH and UCN.

8.2. Methods

8.2.1. Participants

8.2.1.1. Sample

Healthy pregnant women between the ages of 18 to 45 with singleton intrauterine pregnancies were recruited for the study. As AC involves an average complication rate of 0.74% (Bettelheim et al., 2002), only women undergoing this invasive prenatal diagnostic procedure due to karyotyping were eligible for the study. The recruitment took place in cooperation with hospitals in the German speaking part of Switzerland, namely the Departments of Gynecology and Obstetrics at the University Hospital of Zurich, the Hospital of Wetzikon and the Cantonal Hospital of Lucerne from April 2009 to December 2010.

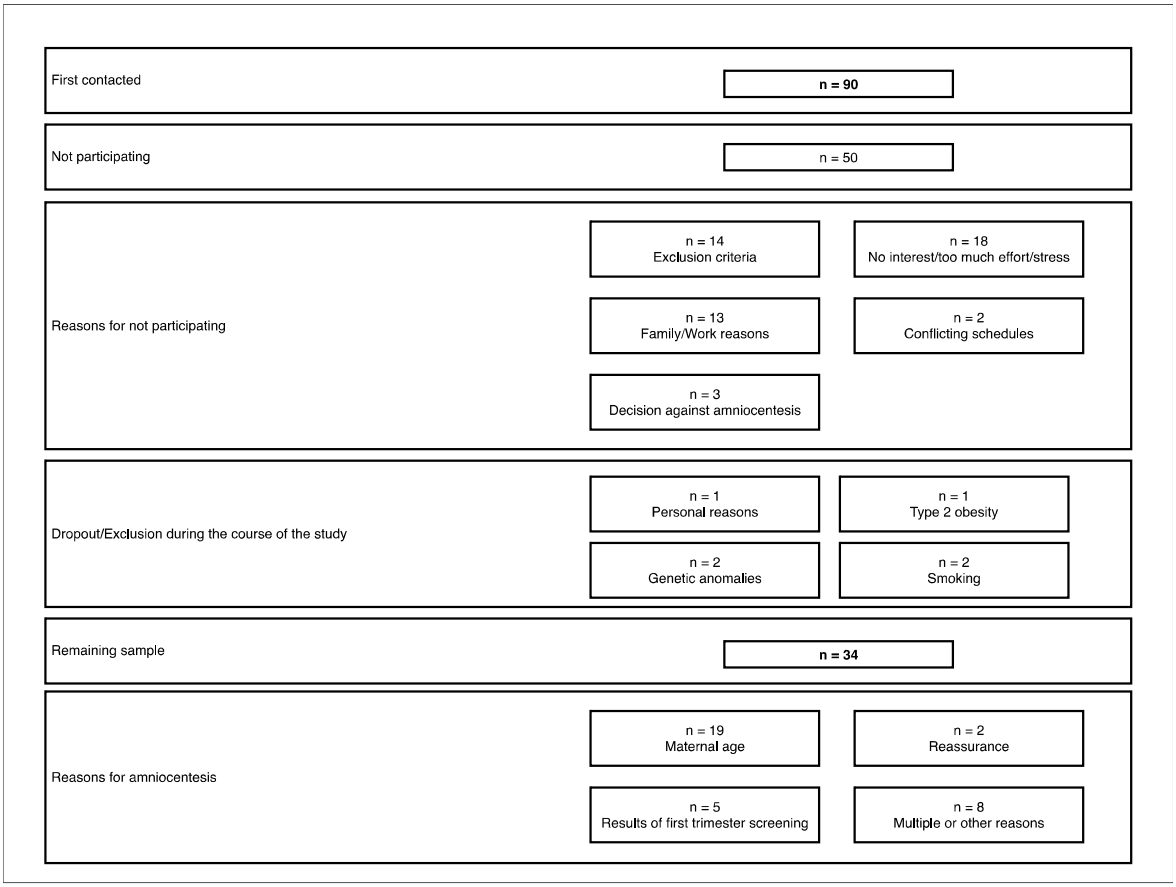
8.2.1.2. Exclusion criteria

Strict exclusion criteria were clarified during a phone screening after the women had been assigned for amniocentesis. Potential participants were excluded due to the following reasons: artificial insemination, known medical complications (concerning the mother-to-be or the fetus), suspected or known fetal growth restriction, ultrasound confirmed fetal structural anomalies, further psychiatric disorders of the pregnant women, smoking and/or alcohol intake, current medication (such as glucocorticoids, psychotropic drugs, diuretics, antihypertensives, vasodilators), food and/or protein restrictions or insufficient language knowledge (because of the study instructions and the questionnaires).

The total recruiting procedures with exclusions and inclusions is pictured in Figure 8.1. Out of 90 women contacted and asked for their participation in the study, 50 women did not participate due to reasons depicted in the figure. In total, 39 healthy pregnant women with singleton intrauterine pregnancies participated in the study. After the study started, 5 women dropped out or had to be excluded: One women cancelled due to personal reasons, two women terminated pregnancy after the fetus was diagnosed with genetic anomalies, two women had to be excluded because of smoking and one women due to type 2 obesity. Therefore, the remaining sample consisted of 34 healthy, pregnant women.

Each woman signed a written informed consent before entering the study procedure. The study protocol was approved by the ethics committees of the canton of Zurich and of the canton of Lucerne.

Figure 8.1. Sampling procedure



Note. The sampling procedure including the reasons for amniocentesis

8.2.2. Procedure

8.2.2.1. Assessment time points

The pregnant women were examined on two different study days. On day 1, the amniocentesis (stress condition) was conducted, while on day 2, 2.7 weeks later (*SD* = 1.00 weeks), a rest condition took place. The rest condition was necessary to control as to whether or not the amniocentesis was subjectively stressful for the pregnant women in our sample, and therefore justified as a standardized stressor in the study. The period of two weeks between the two assessment days was chosen because it was necessary that the pregnant women had received the results prior to this assessment in order to make sure that the rest condition assessment was not influenced by anxiety due to the waiting for the results. Moreover, only women with results without pathological findings were examined throughout pregnancy. Apart from the amniocentesis, the rest condition was identical to the stress condition.

8.2.2.2. Biological assessments

Amniocentesis was performed by the medical doctors in charge of the procedure at the respective hospital. A surplus of 2ml of amniotic fluid was used for study purposes aside from the sample for routine genetic analysis. The amniotic fluid was frozen at -80° C immediately after its withdrawal until chemical analyses took place. Analyses were performed at the Max Planck Institute of Psychiatry laboratories in Munich (Germany) by the Stalla Group.

CRH-levels were estimated with a CRH-radioimmunoassay (RIA) developed by GS. The procedure of the raising of antibodies in rabbits by immunization and the preparation of the CRF are described elsewhere (Stalla et al., 1989; Stalla, Stalla, Schopohl, Werder, & Müller, 1986). The lower limit of detection (LOD) was 40pg/ml. The intra-assay coefficient of variance was 6.1%; the inter-assay coefficient of variance 7.9%.

UCN was analyzed using the RK-019-14 Urocortin (Human) - RIA-Kit (range: 10-1280 pg/ml) by Phoenix Pharmaceuticals, Inc. Karlsruhe, Germany, with a rabbit anti-peptide serum. The general protocol of the exact procedure is readily available (Phoenix Pharmaceuticals Inc., 2011). The lower LOD for urocortin was 20pg/ml.

For all statistical analyses, values below the LOD were set to LOD/√2, following recommendations for not highly skewed data by (Hornung & Reed, 1990). This was the case for 11 CRH values and none of the UCN values.

8.2.2.3. Psychological Assessments

8.2.2.3.1. Stress experience

The stress experienced during amniocentesis (SEA) itself, as well as well as the stress experience caused by waiting for the results (SEW), were assessed using visual analogue scales (VAS). Each response was given by marking a cross on a 100mm line; with a continuum beginning from 0 (*not at all*) to 100 (*highest possible*). The questions concerning amniocentesis included actual subjectively perceived anxiety, stress, arousal, personal challenge and the urge to leave the situation, namely (1) current nervousness, (2) current stress-level, (3) desire to leave the situation immediately, (4) challenge of the situation, (5) current general anxiety and (6) current anxiety of the procedure.. The questions were asked repeatedly at 40, 15 and 1 minutes before the amniocentesis.

A mean score was computed over the different assessments. Additionally, the analyses for the amniocentesis condition were repeated on an item-based level by the dominant item of the cumulated scores which was item (6) current anxiety of the procedure (SEA-1).

The questions assessing the subjective stress experience due to the waiting for the results included (1) anxiety over a possible negative result and (2) disagreeableness of the waiting for the results.

For item-based analyses, both items were regarded separately, using item (1) for the analyses of the stress experience caused by the possibility of a negative result (SENR-1), item (2) for the one item only SEW-1.

The rest condition was scheduled at exactly the same time as the amniocentesis day. To calculate possibly perceived stress during the rest condition, the following items were used: (1) current nervousness, (2) current stress-level, (3) desire to leave the situation immediately, (4) challenge of the situation, and (5) current general anxiety.

8.2.2.3.2. Trait anxiety

Trait anxiety was assessed using the trait version of the German version of the State-trait Anxiety Inventory (STAI) by Laux, Glanzmann, Schaffner, & Spielberger (1981). The items accounting for trait anxiety ask the participant to describe how she generally feels in relation to anxiety. Answers are given on a four-point Likert-type scale ranging from (1) *almost never* to (4) *almost always*. The item scores were summed up for the analyses. Reliability and validity of the STAI are considered to be good (Laux et al., 1981).

To make sure that we could differentiate between the effects of stress due to the amniocentesis and trait anxiety, anxiety was measured in a stress-free rest condition.

8.2.2.4. Statistical Analyses

Statistical Analyses were performed with IBM SPSS Statistics 19 for Mac OS X.

To determine whether or not the amniocentesis was a stressful experience for the pregnant women, perceived stress during amniocentesis and during the rest condition were compared by t-tests.

To analyze the relation of perceived stress and CRH and UCN, high and low perceived stress groups were computed for SEA, SEW as well as the one item-based perceived stress levels by using a median split.

For the analyses of the relationship between trait anxiety measured two weeks after the amniocentesis at the rest condition and CRH and UCN, high and low CRH respectively UCN groups were computed in the same manner.

To compare means, analyses of variance (ANOVA) and t-tests were conducted for all relations with UCN. Inasmuch as the normality of the distribution of CRH after the setting of the values below the LOD (according to Hornung & Reed, 1990) was questionable, Mann-Whitney U Tests were used for the analyses including CRH. Pearson's correlations and Kendall's tau coefficients, respectively, for all study variables were computed.

The level of significance was set at $p < .05$. for all analyses.

8.3. Results

8.3.1. Descriptive analyses

8.3.1.1. Sample

The mean age of the participating pregnant women was 37.4 years, *SD* = 4.0 years. The gestational age at amniocentesis was on average 16.00 weeks (*SD* = 0.71 weeks). Means and standard deviations of all study variables can be found in Table 8.1.

Table 8.1. Descriptives of the subjective stress and anxiety variables as well as CRH and UCN during amniocentesis condition

	N	*M*	*SD*	Range
Stress experience during amniocentesis				
SEA	34	40.66	19.11	2.78-79.75
SEA-1	34	44.80	23.82	0.00-96.17
Stress experience caused by having to wait for the results				
SEW	34	48.98	23.42	3.00-97.19
SEW-1	34	56.88	24.43	3.33-99.33
SENR-1	34	44.28	27.45	2.33-100.00
Stress experience during the rest condition				
SER	34	16.80	12.70	1.80-49.93
Anxiety				
STAI	27	33.90	8.17	21.00-54.29
Biological parameters				
CRH	32	80.33 (pg/ml)	57.24 (pg/ml)	28.28-214.00 (pg/ml)
UCN	32	59.75 (pg/ml)	19.75 (pg/ml)	25.00-100.00 (pg/ml)

Note. Descriptives for all study variables: Stress experience during amniocentesis (SEA) cumulative (as described above) and one item only; stress experience due to the waiting for the results (SEW) cumulative and one item only, stress experience due to the fear of a negative result (SENR) one item only; stress experience during the rest condition; trait anxiety (STAI), corticotropin-releasing hormone (CRH) and urocortin (UCN).

The t-tests revealed no significant differences for the low and high perceived stress as well as for the low and high anxiety groups with regard to maternal age, gestational age and maternal weight. Those possible confounders were therefore not included into the analyses.

A significant correlation was found between CRH and UCN by calculating Kendall's tau coefficient ($r = .258$, $p = .049$).

8.3.2. Amniocentesis condition vs. rest condition

Perceived stress during the rest condition was significantly lower ($M = 16.80$) compared to the amniocentesis condition ($M = 40.66$, $p < .000$). Therefore, amniocentesis could be assumed to be a stressor for our sample.

8.3.3. Stress during amniocentesis

The mean rank of CRH in the low SEA group was 19.24 and 13.40 in the high SEA group. The distribution of CRH given the low SEA group was not significantly different from that given the high SEA group ($U = 81.00$, $p = 0.069$), although a strong trend was visible. When using one item only (SEA-1) to split the sample into high and a low perceived stress due to amniocentesis groups, the distribution showed to be significantly different in the low SEA-1 group (mean rank 19.53) than in the high SEA-1 group (mean rank 13.07), $U = 76.00$, $p = 0.044$.

No such relations were found for UCN levels using a t-test.

8.3.4. Stress caused by waiting for the results

When it came to the stress experience due having to wait for the results, the group perceiving lower disagreeableness concerning the stress of having to wait for the results (SEW-1) showed a significant difference in the distribution of CRH (mean rank 20.07) versus the distribution of CRH in the group that perceived high disagreeableness (mean rank 13.35). The significant difference in the distribution of CRH in the two groups ($U = 74.00$, $p = 0.037$) pointed to lower CRH levels in those women with greater distress due to the waiting period

until they got the results of the amniocentesis.

No such differences were found for urocortin.

8.3.5. Trait anxiety

The mean rank of the CRH-levels in the low trait anxiety group was 16.96, the mean rank of CRH in the high trait anxiety group was 10.81. The distributions were shown to differ significantly (U = 49.50, p = 0.039), indicating significantly reduced CRH levels in pregnant women with increased trait anxiety scores.

Again, no such differences were found for urocortin.

8.4. Discussion

To the best of our knowledge, this study is the first to evaluate the relationships between CRH and UCN in the amniotic fluid and perceived stress due to amniocentesis and anticipating the results as well as trait anxiety in pregnant women.

We found an inverse relation of amniotic fluid CRH with perceived acute stress and trait anxiety but no such relations for UCN with any of the psychosocial parameters, although CRH and UCN did correlate significantly.

Moreover, the results have confirmed that amniocentesis is a stressful event in the life of pregnant women, not only with regard to the procedure itself, but also due to the waiting period until notification of the test results and the feared outcome of the prenatal diagnosis. These findings are in line with previous research, showing that amniocentesis is accompanied by anxiety due to the procedure, but also due to possible negative results (Marteau et al., 1992).

Concerning the relationship of CRH with perceived stress and anxiety, our results may not be easily reconciled with findings from previous research. There are studies investigating such associations of CRH and perceived stress, but with the difference that CRH was assessed

in maternal plasma (Chen et al., 2010; Hobel et al., 1999). Other studies assessed CRH (Florio et al., 2008; Menon et al., 2008; Salminen-Lappalainen & Laatikainen, 1990; Stalla et al., 1989; Torricelli, Voltolini, Galleri, et al., 2009) in amniotic fluid, but did not investigate the association with any psychological variables such as stress or anxiety.

Although negative correlations of CRH and perceived stress might appear surprising at first sight, it has to be noted that, during pregnancy, the relationships between stress biomarkers and perceived stress has to be re-evaluated, as a recent review was able to show that the usual connections seem not to be found in this phase of a woman's life (Harville et al., 2009). Correlations reported for CRH and psychosocial measures in pregnant women were no larger than .15. The authors hypothesized, therefore, that a more detailed measurement of the biomarkers would be necessary; concerning CRH this would mean a closer look to the CRH-binding protein as well and the separate inputs of placental and hypothalamic CRH. Several other studies support these findings by detecting neither a relationship between maternal plasma CRH and perceived stress (Himes & Simhan, 2011; Kramer et al., 2009; Petraglia et al., 2001) nor an increase of CRH release into fetal plasma as a reaction to the stress of an invasive medical procedure (Gitau, 2004). Hobel et al. (1999) reported a diverging association of CRH and stress in a preterm group (positive associations) versus a group with term deliveries (negative associations). All these studies referred to maternal plasma CRH levels, which are notably higher (up to 20- to about 30-fold measured in the third trimester) compared to amniotic fluid levels (Salminen-Lappalainen & Laatikainen, 1990; Stalla et al., 1989). Petraglia et al. (2001) suspected that they did not detect a connection between serum CRF and psychosocial stress due to the anyway high levels of (placental) CRH during pregnancy.

In our study, we found a significant inverse correlation of trait anxiety and amniotic fluid CRH. Harville et al. (2009) also considered state and trait anxiety in their review. They did find a slightly negative correlation, which was not statistically significant. There was also no significant correlation of pregnancy-related anxiety in their database of 1587 women. In contrary to that, Mancuso et al. (2004) found a positive correlation of pregnancy specific anxiety and CRH during the third trimester, but not during the second trimester; while they did not find any connection with state anxiety and CRH at all.

Other studies connect altered levels of maternal CRH levels to personal factors rather than

to stress related variables. Coping styles like disengagement and religion seemed to be protective as well as a longer duration of sleep per night (Latendresse & Ruiz, 2010). Furthermore women from Sub-Saharan Africa or the Caribbean or with normal weight/less likelihood to obesity (Kramer, Lydon, Séguin, Goulet, Kahn, McNamara, et al. 2010) seemed to be connected with lower levels of CRH during pregnancy. Pregnant women with higher CRH levels indeed seemed more likely to be smokers (Kramer et al., 2010).

The findings that UCN was not related with any of the stress and anxiety measurements might also be viewed in the context of the role of UCN in stress-recovery found in animal studies (Neufeld-Cohen et al., 2010), indicating that the lack of urocortin does not show alterations in the immediate stress response, but prevents adaptive stress recovery. The principal involvement of UCN therefore might be in the (successful) adaptation to stress (see also Joëls & Baram, 2009). Still, the role of UCN in the human stress response is not fully understood due to its intermingling actions through both CRH-1- and CRH-2-receptors (Bale & Vale, 2004). Further studies have to thoroughly investigate not only its role in the human stress response, but also in human pregnancy to discover possible pathways of how stress biomarkers are related to stress experience in this specific phase of life.

8.4.1. Limitations of the study and implications for further research

Apart from larger sample sizes when aiming to replicate the results presented here, the simultaneous assessment of CRH and UCN in maternal plasma as an addition to the corresponding amniotic fluid levels would be highly recommended. The relations between serum and amniotic fluid concentrations are still poorly understood and require further evaluations. In this connection, the observation of the other CRH-family peptides, UCN-2 and UCN-3 could receive focus as well, to elucidate the different pathways and the role of the CRH-1- and CRH-2-receptors.This could be instrumental in exploring the functioning pathways of the impact of prenatal maternal stress.

High attention should be paid in the selection of the technique for the biochemical analysis of CRH and UCN. The existing studies measuring the peptides in amniotic fluid in healthy pregnancies show an unusually wide range of measured levels of CRH (Florio et al., 2008; Menon et al., 2008; Salminen-Lappalainen & Laatikainen, 1990; Stalla et al., 1989; Torricelli,

Voltolini, Galleri, et al., 2009) and UCN (Iavazzo,Tassis, et al., 2009; Torricelli, Voltolini, Biliotti, et al., 2009;, Torricelli, Voltolini, Galleri et al., 2009). This issue might be critically important to account for in future studies as the range of levels reported in current research have been oddly divergent. Concerning CRH, the studies comparable to our study considering age of gestation were the following: Salminen-Lappalainen and Laatikainen (1990) measuring time point 15-17 weeks of gestation, used a radioimmunoassay (RIA) and reported a mean level of 44.25 pg CRH per ml (*SD* = 4.28 pg/ml). Torricelli, Voltolini, Galleri, et al. (2009) assessing at a mean gestational age of 16.19 weeks used a quantitative colorimetric immuno-assay and found 1640 pg CRH per ml (*SD* = 680 pg/ml). In our study, at 15.5 weeks of gestation, using a RIA, 84.20 pg of CRH per ml were measured with a standard deviation of 58.63 pg/ml. These results show that the same method of analyses, in this case RIA, resulted in levels of a similar range.

The UCN levels in studies comparable considering the week of gestation varied: Iavazzo, Tassis, et al. (2009), assessing at 15.9-23.7 weeks of gestation, used an enzyme-linked immunoabsorbent assay (ELISA) and reported 1600 pg UCN per ml (*SD* = 490 pg/ml). Torricelli, Voltolini, Biliotti, et al. (2009), also assessing the peptides in the second trimester at 15-16 weeks of gestation, used a specific and sensitive immunoenzymatic assay and found 900 pg UCN per ml (*SD* = 270 pg/ml) and in another study of the same research group (Torricelli, Voltolini, Galleri, et al. 2009), measured at 16.19 weeks of pregnancy with a specific and sensitive immunoenzymatic assay, 900 pg UCN per ml (*SD* = 260 pg/ml) were reported. Our UCN levels, measured at a mean gestational age of 15.50 weeks, using a RIA, add up to 60.03 pg/ml with a standard deviation of 20.15 pg/ml. None of the other studies measured UCN with a radioimmunoassay. Clifton et al. (2000), have already addressed this issue, pointing to the different techniques used for the biochemical analyses in current research and the resulting difficulties involved in trying to compare the results.

Another issue is the relatively high LOD of both UCN and CRH. Whether the values below these specific LODS are included in the statistical analysis or not and, if so, the procedure of how these values are handled in the statistical analyses, should be documented in all future publications, because diverging approaches could add to the unwanted variety in reported peptide levels.

8.4.2. Perspectives

The newly detected relationships among stress-related peptides in amniotic fluid might help to analyze the pathways on how stress, peptides involved in the stress reaction and pregnancy and birth complications fit together. The findings highlight the importance of assessing psychological variables associated with stress – along with stress related peptides during pregnancy.

Part IV. General Discussion

9. General discussion

In the following, the experimental studies will be discussed with respect to their integration in the current literature, the research approach and its possible limitations, directions for future research and implications for clinical practice.

9.1. Summary of the experimental studies with integration into the current literature

As it has been shown, stress, anxiety and depression may have a major impact on the course and outcome of pregnancy. What is still poorly understood are influencing factors and resources of resilience as well as the biological pathways of the effects. The main aims of the experimental studies described above was to contribute to the elucidation of this complex field by the investigation of important parameters in the amniotic fluid and to point to possible protective factors that could be used in preventive programs by looking at the way pregnant women handle adversities during pregnancy.

9.1.1. Study 1 - Coping styles in relation to perceived stress, birth anxiety and depressive mood during pregnancy

In the first study, the effect of certain coping styles, their possible predominance and stability or change in the principal coping styles respectively, have been studied in relation to acute perceived stress due to prenatal diagnosis. Furthermore, its possible consequences as well as the connection to depressive mood and birth anxiety were examined.

Interestingly, most women predominantly showed a task oriented coping style at all assessment time points. While task oriented coping style is generally seen as protective in situations where the individual is in full control, it is seen as possibly harmful in uncontrollable situations. Therefore it seems surprising that the pregnant women in our sample also mostly showed predominant task oriented coping strategies. Though, Folkman & Moskowitz (2000) have stated and shown that this theoretical assumption might be oversimplified in a way,

bearing in mind that superficially uncontrollable situation still do have controllable aspects and therefore might be protective in such situations as well. The finding that the means of task oriented coping were highest during the second as well as in the third trimester goes along with previous studies using the CISS during pregnancy (i.e. Da Costa, 1997) and studies not using the CISS, but measuring similar concepts such as planning and preparation, i.e. Hamilton & Lobel, (2008) or task coping measured with other instruments (Borcherding, 2009).

It has been shown that a higher use of emotion oriented coping predicts higher perceived stress during amniocentesis, while more use of avoidance focused coping strategies is related to higher perceived stress due to the waiting for the results. A predominant task oriented coping style during the third trimester of pregnancy seemed to have a protective effect, in a way that pregnant women who predominantly used these coping strategies showed lower levels of depressive mood and birth anxiety. This specific finding is even more interesting considering the fact that most of the women used task coping predominantly throughout the course of the study, i.e., the early second trimester until the third trimester. This shows that our sample mostly showed healthy and benefitting coping strategies during this sensitive phase of life, impacting their own lives and the lives of their unborn children.

Independently of the predominant coping style, the use of more emotion-focused coping strategies was related to higher depressive mood and birth anxiety during the third, but not the second trimester of pregnancy. The result that higher levels of EC go along with higher levels of depressive mood is in line with previous research (Da Costa, 1997; Da Costa, Larouche, et al., 2000).

The change of the coping style at any time during the study period accounted for higher levels of depressive mood only during the second trimester. As neither of the changes between specific time points showed a significant relation with either depression or birth anxiety, timing and whether the change is a precursor or a consequence of depressed mood can not be evaluated. Further research is therefore necessary to examine the possible benefit or harm of the change of predominant coping styles during pregnancy.

9.1.2. Study 2 - Second trimester amniotic fluid corticotropin-releasing hormone and urocortin in relation to perceived stress and anxiety during amniocentesis

The second study had as its primary aim the evaluation of urocortin and corticotropin-releasing hormone levels in connection with perceived stress due to amniocentesis and, again, possible negative results as well as trait anxiety in the second trimester of pregnancy.

The results clearly confirmed a finding of previous studies of other work groups to the effect that amniocentesis – with regard to both the procedure itself and the waiting for the results - is a stressful event for pregnant women (Brajenović-Milić et al., 2010; Marteau et al., 1992).

To our knowledge, this study was, moreover, the first to characterize the relationships between CRH and UCN in the amniotic fluid and perceived stress and trait anxiety. The finding that CRH in amniotic fluid is negatively associated with perceived stress and trait anxiety might be surprising, as it is an important hormone in the human stress response and is secreted into the circulation in notable amounts following exposure to a stressor. It has to be noted though that several studies examining plasma CRH in pregnancy found a significant relationship between these levels and perceived stress neither in pregnant women (Himes & Simhan, 2011; Kramer et al., 2009; Petraglia et al., 2001) nor in fetal plasma (Gitau, 2004). A relatively new review even suggests a reconsideration of the common biomarkers of stress when it comes to studies during pregnancy (Harville et al., 2009).

9.2. Summary of the possible limitations of the experimental work

During the conception, design and preparation period of our experimental work as well as during the assessments, we did our best to avoid any known or possible pitfalls to the best of our knowledge. Nevertheless, as in any experimental study, the accomplishment and the findings implicate possible limitations of the work, which will be discussed in the following.

9.2.1. General Limitations

General limitations of both studies will be described in the following, before the individual details of both studies will be discussed.

9.2.1.1. Sample size

Due to the strict exclusion criteria and the fact that the participating women had to undergo an amniocentesis for medical reasons or reasons of age or interest, the recruitment for the study was very challenging. Total recruiting time was about 1.5 years, with an outcome of 39 participants. About 45.5% of the women initially asked to participate finally did enter the study, thus that the number of possibly eligible women was already small, despite the fact that it was a multicentric study. For future studies, it would be highly desirable to acquire larger sample sizes, which is in Switzerland – given the size of the hospitals and the number of patients treated in each of them – only reachable if more hospitals across the country would participate in the study.

9.2.1.2. Dropouts

The problem of attrition is, of course, a general problem, especially, however in studies with already small sample sizes. In our study, the time and effort that the participating women had to put in, was very high, and it seems likely that this may have contributed to the high number of dropouts. The most pronounced lack of data can, as would be expected, be seen in the third trimester, where questionnaires had to be filled out online or by hand at home. For future studies, in-house appointments at all assessment time points could help to lower the data loss.

9.2.2 Limitations of Study 1 - Coping styles in relation to perceived stress, birth anxiety and depressive mood during pregnancy

9.2.2.1. Homogeneity of the sample

Our study sample was very homogenous with respect to age, socioeconomic status and educational background. The distributions of those variables were so well balanced, that it was not necessary to control for these factors, which are potential confounders when it comes to

coping strategies. While this high comparability is an advantage on the one hand, on the other hand, the homogeneity was not only limited to sociodemographic variables: Levels of depressive mood and birth anxiety also appeared to be relatively low across the entire sample. For future studies, a more heterogeneous sample would be desirable.

9.2.2.2. Operationalization of the study variables

The problems of measuring and operationalizing coping during pregnancy have already been described above in Chapter 7. There is an ongoing debate concerning whether to use pregnancy specific or general coping assessments. There are pro and contra arguments for both sides: Pregnancy specific tools do have the advantage of fully capturing the particularities of this phase in the life of a woman. General coping tools have the advantage of being able to compare general coping in different life phases independently. Future studies would do best in assessing both pregnancy specific and general coping strategies.

9.2.3. Limitations of Study 2 - Second trimester amniotic fluid corticotropin-releasing hormone and urocortin in relation to perceived stress and anxiety during amniocentesis

9.2.3.1. CRH and UCN – where to assess for best results?

It has been shown in Chapter 2, that, so far, most studies in the field of pregnancy assessed CRH in maternal plasma or placental tissue, while UCN-assessing studies mostly focused on amniotic fluid. The possibilities to assess the peptides in amniotic fluid are ethically limited, as every amniocentesis bears a risk for mother and fetus. In our study, we were especially interested in the amniocentesis procedure as a standardized biological (the trans-abdominal puncture) and psychosocial (fear of the results, fear of the procedure with its possible risks) stressor, which gave us the possibility of measuring the peptides in amniotic fluid that was destined to be sampled for genetic screening in the sense of prenatal diagnostics anyway.

In future studies, the assessment of both maternal plasma and amniotic fluid levels should be carried out. This could lead to a major contribution in the goal of reaching a better understanding of the signaling pathways.

9.2.3.2. CRH and UCN – biochemical analyses

The levels of CRH or UCN measured is highly dependent on the methodology and assay used by the analyzing laboratory (Clifton et al., 2000). This makes it either complex or nearly impossible to compare the few existing studies on the peptide levels in amniotic fluid and to integrate one's own data, as described in Chapter 2.3.3 and Tables 2.2 and 2.3. In future studies, highest attention has to be paid to the chosen assays and to the completion of the analyses. Whenever possible radioimmunoassays should be used for more accurate results.

Another issue is the handling of values below the limit of detection of the peptide. The procedure used should be accurately described. A well-known and often used procedure applicable for small sample sizes with either skewed or non-skewed data is described by Hornung and Reed (Hornung & Reed, 1990).

The above-mentioned issues and possible limitations were addressed in our study. What limits our result in some way, is the lack of comparability due to the high variation in biochemical and statistical procedures used in previous research.

9.3. Directions for future research and clinical implications

As a final résumé, the clinical implications and directions for future studies drawn from our experimental studies will be summarized. While the effects of stress, anxiety and depression during pregnancy are well documented by previous research, uncertainties still abound. There are voices claiming that existing research is limited in comparability and generalization because of the chosen study samples and the wide variety of operationalization and assessment instruments of prenatal stress (Beydoun & Saftlas, 2008). Ayers (2001) also pointed to the difficulty of measuring stress and coping in pregnant women and new mothers, as the concepts are broad and across pregnancy, overlapped with pregnancy-specific factors. Other authors even doubt the adequacy of well-studied biological stress markers during pregnancy, as they were not necessarily related to perceived stress in their study (Harville et al., 2009). A possible explanation could be, that the stress-related hormones do play a key role in the course of pregnancy as well, factors that make it difficult to filter out the overlap.

Altogether, the signaling pathways still remain mostly unclear and many possibly influencing factors have not been investigated yet. This underlines the necessity of future studies, as it will be advised in the following, in addition to the issues already addressed in the limitation section, Chapter 9.2.

9.3.1 Directions and implications: Study 1 - Coping styles in relation to perceived stress, birth anxiety and depressive mood during pregnancy

The fact that various studies indicate a mostly negative impact of biological and psychosocial stress during pregnancy – including long-term effects for the well-being of mother and child – is critically important. The mere thought that stress, a factor that is barely escapable in nowadays society, might have at least as severe impacts on pregnancy and the child as known evitable factors such as smoking, alcohol and illegal drug intake or certain nutritional factors such as rare meat or fish, is frightening at first.

It's good to know that there are, in fact, antidotes: Pregnant women, also those in the high-stress groups, profit from stress management programs, education about stress and relaxation techniques (Ickovics et al., 2011; Urech et al., 2010; Urizar et al., 2004). Moreover, some women seem to have some kind of natural protection, a resilience against the effects of stress – namely strong personal resources in general (Nierop et al., 2008; Rini et al., 1999) or, more specifically, higher levels of self-efficacy (Nierop et al., 2008). Our first study has shown that also the strategies pregnant women use to face acute stressful situations may influence the effect that the stressor has on her well-being during pregnancy. It has been shown that the use of task oriented coping strategies seem to have a protective value, whereas emotion and avoidance oriented coping is related with higher stress in specific situations. Moreover, a more frequent use of EC in stressful situations was also related to lower well-being across pregnancy. This knowledge could and should be integrated into educational programs for pregnant women, in order to teach them the value of task oriented coping strategies and to show them alternatives to – for this phase of life – dysfunctional ways of handling stressful situations.

Inasmuch as coping in acute stressful situations and especially pregnancy-specific stress is still poorly understood, future studies should take this into account, along with chronic stress and daily hassles in general.

9.3.1. Directions and implications: Study 2 - Second trimester amniotic fluid corticotropin-releasing hormone and urocortin in relation to perceived stress and anxiety during amniocentesis

The biological pathways of the effects of stress on the course and outcome of pregnancy are still poorly understood. Aside from the direct effect of stress-related hormones on fetal neurodevelopment during pregnancy, current research focuses on the so called fetal programming of the HPA, a system that involves permanent and probably dysfunctional alterations of the stress response system, with lifelong effects for the offspring. Therefore, it is more than necessary to get a better understanding of all HPA hormones and their presence and release in human pregnancy.

Our second study looked at two of those hormones, namely CRH and UCN in the closest environment to the fetus – the amniotic fluid. As to our knowledge, our study was the first to investigate the amniotic fluid levels of these hormones with perceived acute stress and anxiety in pregnant women. Further studies with preferably larger sample sizes will be necessary to confirm our findings. We did find a negative correlation between amniotic fluid CRH and perceived stress in the pregnant mother-to-be. There are some studies that have investigated this association in maternal plasma, though it has to be noted that plasma CRH levels are much higher than the concentrations in amniotic fluid (Salminen-Lappalainen & Laatikainen, 1990; Stalla et al., 1989). Several studies, so far, have not found a relationship between maternal plasma CRH and perceived stress during pregnancy (Himes & Simhan, 2011; Kramer et al., 2009; Petraglia et al., 2001); a stressor-related increase was likewise not detectable (Gitau, Fisk, Teixeira, Cameron, & Glover, 2001). In future studies, it would, therefore, be very interesting to assess plasma and amniotic fluid CRH and UCN levels simultaneously to get a better understanding of stress-response release-patterns during pregnancy.

Part V: Appendix

10. References

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Curriculum vitae

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1989-1994	Primarschule (elementary school) in Teufenthal
1994-1998	Bezirksschule (junior high school) in Unterkulm
1998-2002	Neue Kantonsschule Aarau (high school), Department of Modern Languages
2002-2003	Studies in information systems, economics and private law at the University of Zurich
2003-2007	Studies in psychology, psychopathology and public health at the University of Zurich
2006	Internship as clinical psychologist in the psychiatric hospital Klinik St. Urban LU
2007	lic. phil. in psychology, equivalent of M Sc UZH in psychology
2008	Research scholar at Harvard University (USA), Affective Neuroscience Laboratory of Prof. Dr. Diego Pizzagalli
2008-2011	Doctoral student at the University of Zurich, Institute of Psychology, Department of Clinical Psychology and Psychotherapy, Prof. Dr. Ulrike Ehlert
2008-today	Further education in cognitive behavioral therapy and behavioral medicine in order to earn the degree of Master of Advanced Studies in Psychotherapy at the University of Zurich
2009-2011	Counseling psychologist at the University Hospital of Zurich, Department of Obstetrics and from 2010 on in the Department of Neonatology
2010-2014	Therapist at the outpatient unit for Cognitive Behavioral Therapy and Behavioral Medicine, Centre of Psychotherapy, University of Zurich
2011-2013	Clinical psychologist at the Rehaklinik Bellikon, Department of Work-Oriented Rehabilitation
2012	Defense of doctoral thesis
2013-today	Advanced studies in clinical hypnosis and hypnotherapy at the Gesellschaft für Klinische Hypnose Schweiz (Society of Clinical Hypnosis Switzerland, ghyps)
2013-today	Delegated psychotherapist at the Praxis am Central, psychiatric-psychotherapeutic group practice, Zurich, medical director Roman Buxbaum MD